A STUDY OF SIMULTANEOUS TRACINGS FROM THE APEX OF THE HEART AND THE RADIAL ARTERY WITH THE MICROGRAPH.*

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The general method of producing tracings of the pulse by the use of the principle of light interference, and some representative curves obtained in this way have been given in a former communication.[1] An instrument has now been designed to furnish two simultaneous tracings, as the original instrument could produce but a single record. This has made it possible to identify with certainty some events about which there was doubt when single tracings only could be taken.

Since the first tracings were made with the micrograph, those from the apex of the heart have attracted our attention especially, because of two characteristic wave-groups of short period that are invariably found, when present at all, at certain definite positions in the cardiac cycle.

Apex tracings from several different subjects are reproduced in text-figure 1, the curves being numbered respectively, 2, 5, 27, 29, 34, and 35. Each apex tracing was taken simultaneously with a tracing from the right radial artery, and the vertical line, RR, in the center of the figure, represents the time of the beginning of the upstroke of the radial curve in all cases, although only two radial curves are shown in full; namely, Nos. 2 and 34.

The speed of the film was approximately six inches per second for all tracings shown in text-figure 1, but because the pulse rates of the subjects differ considerably, the cardiac cycles in the various curves are completed at different times. The time of the beginning of the upstroke of the simultaneous radial curve is indicated for each apex tracing by the vertical lines, R_2, R_5, etc.

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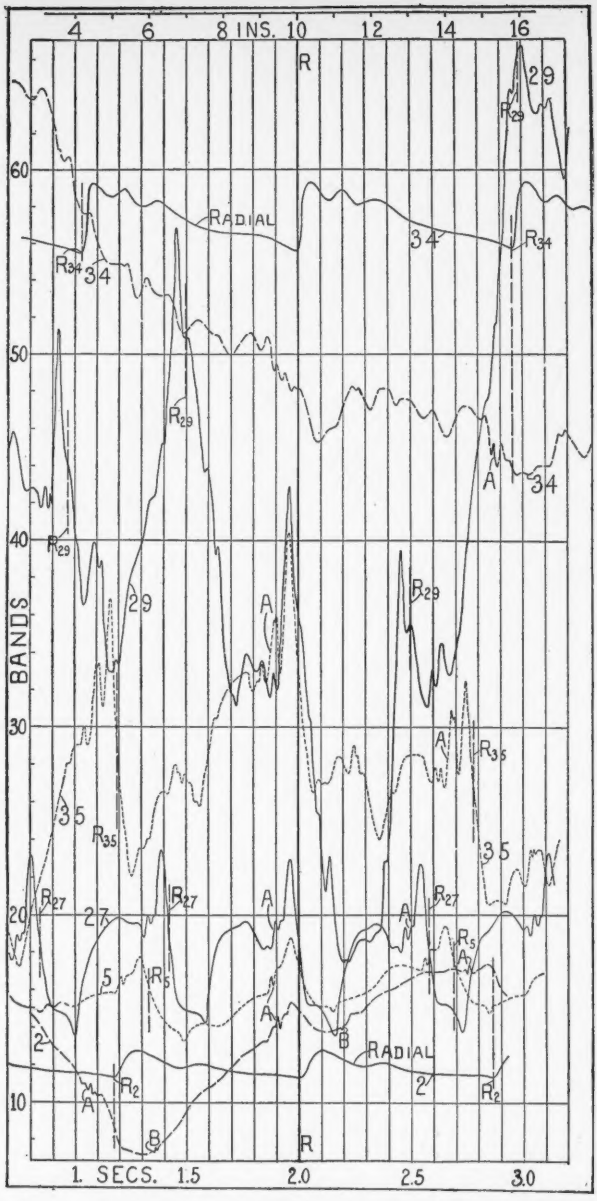
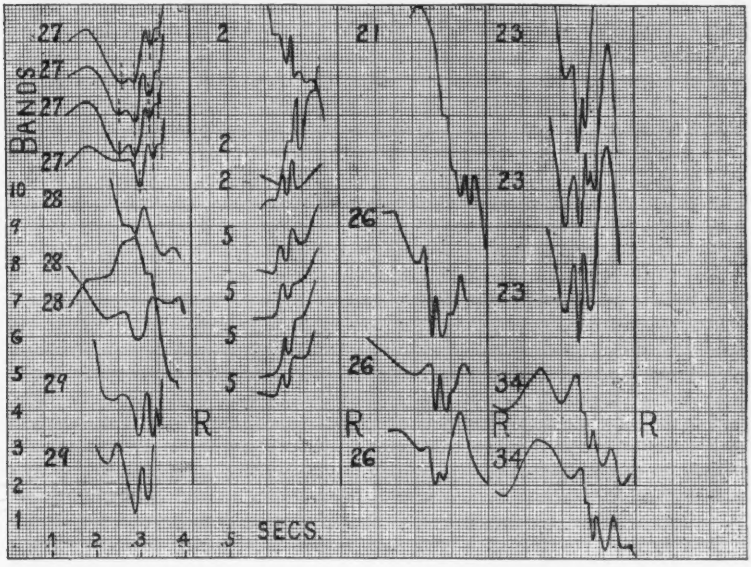
[1] Crehore and Meara, The Micrograph, *Jour. Exper. Med.*, 1911, xiii, 616.



TEXT-FIG. 1. Simultaneous tracings from the apex of the heart and the right radial artery. Nos. 2, 5, 27, 29, 34, and 35 refer to different subjects. The same apex tambour was used for each subject, and the speed of the film was six inches per second. Nos. 5 and 27 are from normal hearts on the same scale (one band per half inch) as the curve No. 29 from an abnormal subject. RR is a vertical line denoting the time of beginning of the upstroke of the radial curve taken simultaneously with each apex tracing. R_2, R_5, etc., mark the times of the preceding or following upstrokes of the radial. The vertical lines mark one tenth second intervals. The A-wave-group is at A one tenth of a second before RR, and the B-wave-group at B, two tenths of a second after RR. The tracings are selected to show the diversity of form rather than similarity.

The positions of the A-wave-groups are clearly seen in these tracings at the points A, and those of the B-wave-groups, when clearly marked, at B. Only two such groups are found in each cycle; the first at A is always more pronounced both as to intensity or amplitude and as to duration than the second group at B. In some subjects the B-group is absent altogether, probably because the amplitude is too small to be recorded at the position of the tambour, or because the cause of it is less marked in the subject. The A-group, on the other hand, is always found in the tracings.

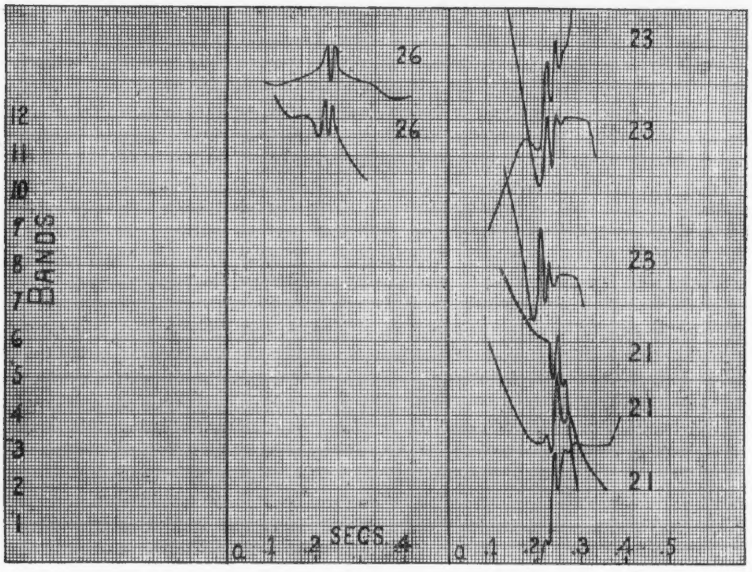


TEXT-FIG. 2. Showing A-wave-groups taken from tracings at the apex of the heart simultaneously with tracings from the right radial artery. The beginning of the upstroke of the radial occurs at a time marked by the vertical lines R. Curves having the same number, *e. g.* 27, are taken from different cycles in the same record from one subject. The curves are superposed in order to show the coincidence in time of the waves from different cycles and subjects. Note that the A-groups appear before the lines R, the beginning of the upstroke of the radial.

A comparison of the apex tracing with the simultaneous tracing from the right radial artery shows that the A-wave-group always occurs a short time before the beginning of the upstroke of the radial curve, and when the B-group is present it always occurs after the upstroke by a different interval of time.

The A-wave-groups have been copied from a number of apex

tracings and are reproduced in text-figure 2, one curve being placed immediately under another for comparison; the B-wave-groups are similarly reproduced in text-figure 3. In each figure the vertical lines, R, give the time of the beginning of the upstroke of the radial curve taken simultaneously with the apex tracings from which the wave-groups are taken. The A-wave-groups occur in all cases before the lines R, and the B-wave-groups after these lines. The apex trac-



TEXT-FIG. 3. Showing B-wave-groups from tracings taken at the apex of the heart and superposed similarly to the A-wave-groups in text-figure 2. Note that these groups appear after the lines R, the beginning of the upstroke of the radial.

ings from successive cycles in the same record from one subject have the same number; for example, the four tracings, 27, in text-figure 2, were taken from the different cycles in the record of one man.

Thus arranged, the tracings show clearly that the A-wave-group occurs with great regularity in different subjects and at practically the same portion of each cycle,—shortly before the upstroke of the radial pulse. The B-wave-groups also occur with the same regu-

larity in different subjects, each about the same time interval after the upstroke of the radial. This is in spite of the fact that the pulse rates in the different subjects differ materially.

The duration of either of these wave-groups, A or B, is sufficiently great to make it necessary in measuring time intervals from them to specify the portion of the wave-group from or to which measurements are made. If the physiological cause of each or any of the component waves making up either of these wave-groups were known with certainty, it would be more satisfactory to measure time from these known events. The plan adopted here is to select the approximate middle point of the group and measure from that point.

Some of the results of such time measurements on different subjects are given in table I. The number of the subject is given in

TABLE I.

Time intervals obtained from synchronous tracings from the apex of the heart and the right radial artery. Column 6 gives the time interval between the A- and the B-wave-groups.

1	2	3	4	5	6	7	8	9
2	73.	.82	.100	.183	.283	.54	.34	.66
5	90.	.67	.121	.158	.279	.39	.42	.58
10	77.	.78	.103					
21	69.	.87	.073	.245	.318	.55	.37	.63
23	70.	.86	.120	.225	.345	.51	.40	.60
26	61.	.98	.111	.232	.343	.64	.35	.65
27	103.	.58	.100	.158	.258	.33	.44	.56
28	72.	.83	.100	.223	.323	.51	.39	.61
29	120.	.50	.108					
30	68.	.88	.087	.250	.337	.54	.38	.62
31	82.	.73	.103	.250	.353	.38	.48	.52
33	90.	.67	.104	.230	.334	.34	.50	.50
34	63.	.95	.100	.234	.334	.62	.35	.65
35	75.	.80	.100	.250	.350	.45	.45	.55
a	80.	.75			.260	.49	.35	.65
b	75.	.80			.290	.51	.36	.64
c	82.	.73			.271	.46	.37	.63
Average			.1021	.2198	.3125			

the first column, the pulse rate, obtained from three or four cycles, in the second column, and the time of one complete cardiac cycle in the third. The time interval from the middle of the A-wave-group to the beginning of the upstroke of the right radial pulse is in the

fourth column. The fifth column gives the time from the right radial to the middle point of the B-wave-group. The sixth gives the sum of the numbers in the fourth and fifth columns and is the time from the middle point of the A-wave-group to the middle of the following B-wave-group. The seventh gives the difference between the intervals in the third and sixth columns and denotes the time for the completion of the cardiac cycle, measuring from the middle of the B-wave-group to the middle of the following A-wave-group. The eighth gives the ratio of the sixth to the third columns, and expresses the proportion which the time interval from the A- to the B-group bears to the whole cycle. The ninth column similarly expresses the ratio to the whole cycle of the interval from the B- to the following A-wave-group, the sum of the numbers in columns 8 and 9 being unity and denoting a whole cycle.

The time required for the blood pressure impulses to travel in the arteries from the left ventricle of the heart to the right radial artery has been measured by numerous observers, and is well known to be approximately one tenth of a second, although the rate of transmission varies considerably in different individuals. As bearing upon this point, a simultaneous record of the radial and brachial arteries in the right arm of one subject was taken, and the time of travel between these arteries was found to be 0.045 seconds. The distance was approximately thirteen inches, and the rate of travel, therefore, was, in this case 289 inches or 7.35 meters per second. The distance from the left ventricle to the right radial artery is somewhat more than double the distance from the brachial to the radial, and the time required to travel from the ventricle to the right radial was therefore somewhat more than $2 \times 0.045 = 0.09$ seconds; that is, not far from one tenth of a second.

THE A-WAVE-GROUP.

Measuring backward a tenth of a second from the beginning of the upstroke of the radial curve, to allow for the time of travel, brings us in every case somewhere within the limits of the A-wave-group. Since the upstroke of the radial pulse is caused by the sudden influx of blood into the radial artery, and this is due to the contraction of the left ventricle of the heart, which almost immediately

opens the aortic semilunar valve and admits blood into the systemic arteries; and since the time of travel of the wave of blood pressure has been found on measurement to be about one tenth of a second, and since in measuring backward a tenth of a second from the beginning of the upstroke of the radial, we come to a point within the limits of the A-wave-group; for these reasons it is safe to conclude that the time of the opening of the aortic semilunar valve is in every case somewhere within the A-wave-group in the apex tracings.

Having conclusively located the approximate point in the apex tracings when the aortic semilunar valve opens, certain deductions about the other valvular events may be made. As there are two valves each in the right and left ventricles of the heart, and each of these valves opens and closes once during a complete cardiac cycle, there must be eight separate valvular events taking place in the ventricles during one cycle, and in the nature of things these events are divided into two groups of four each. These two groups must necessarily be separated by a considerable portion of one cycle, while the four events of each group should happen almost at the same time or in comparatively rapid succession. These statements may safely be made from a knowledge of well known mechanical principles applicable to pumps. The four valvular events composing the first of the two groups are associated with the time of the sudden contraction of the ventricles at the beginning of systole. The state of the valves just prior to this time is as follows: the tricuspid and bicuspid valves are open, admitting blood into the ventricles from the auricles, and the two semilunar valves are closed, preventing a back influx of blood from the systemic and pulmonary arteries. When contraction of the ventricles begins, it would soon have the effect of closing the tricuspid and bicuspid valves—if there were no other cause closing them earlier—and of opening the semilunar valves, the closure of the former preventing back-flow into the auricles, and the opening of the latter admitting blood to the arteries. There probably is a brief moment of time between the closing of the tricuspid and bicuspid valves and the opening of their corresponding semilunars, accounted for by the fact that it requires a greater pressure to open the semilunar valves because of the opposing force of blood in the arteries, than it requires to close the

tricuspid and bicuspid valves. But during this time the ventricles are completely closed chambers filled with blood, and the interval must be very brief.

Since it has been shown that the opening of the aortic semilunar valve occurs during the A-wave-group, and since the closure of the tricuspid and bicuspid valves and the opening of the pulmonary semilunar valve are closely associated in time with the opening of the aortic semilunar valve, it is safe to conclude that the A-wave-group represents the effects produced upon the apex of the heart by this first group of four valvular events within the ventricles.

THE B-WAVE-GROUP.

When systole has begun and the four valves of the ventricles have assumed the positions stated, namely, when the tricuspid and bicuspid valves are closed and the semilunars open, the valves will remain in their respective positions for a considerable portion of a cycle, during which the flow of blood into the arteries takes place; that is, the time is long when compared with the intervals between events in the first group. When diastole of the ventricles sets in, the second group of valvular events takes place, the two semilunar valves closing, and the tricuspid and bicuspid valves opening.

The average duration of systole is known to be not far from three tenths of a second, as measured by different observers, and as the beginning of systole has been shown to be associated with the A-wave-group or first group of valvular events, the second group of four valvular events should occur about the end of systole, nearly three tenths of a second later. In the table, the sixth column shows that the B-wave-group occurs approximately by this interval of time after the A-wave-group.

It is safe to say, therefore, that the B-wave-group occurs at the end of systole and that it includes the time of closing of the semilunar valves, and corresponds to the second group of valvular events above described. Whether or not there are any evidences in the B-wave-group of the opening of the tricuspid and bicuspid valves is not known. The disturbance which they should cause at this time would naturally be less pronounced than the disturbance made when these valves are closed during the A-wave-group, be-

cause they now open under the low pressure of diastole, and they close then under the high pressure of systole. The fact that the B-wave-group is always less pronounced both in amplitude and duration supports this reasoning.

On the assumption that these A- and B-wave-groups in the apex tracings are caused by the action of the valves in the ventricles, as has been shown, the sixth column in the table represents the approximate time of systole. This is well known to be much more constant than the time of the remainder of the cycle in column 7; that is to say, when a variation in the pulse rate occurs, the greater part of the variation occurs in the rest of the cycle included between the B-group and the following A-group.

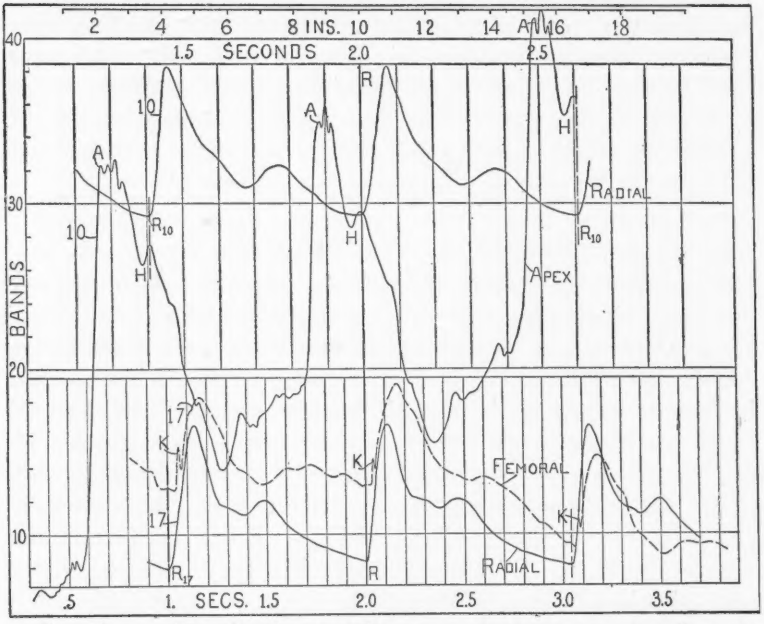
TIME-INTERVALS WITHIN A GROUP.

It is hoped that some time it may be possible to identify some of the individual events in the wave-groups of the apex tracings; but a study of the small wavelets composing an A-wave-group, for example, No. 27, text-figure 2, shows that each little ripple is repeated faithfully in several different cycles. Small vertical marks are drawn in the case of the four curves, 27, at the bottom of each wavelet, and all of these marks are repeated in each curve, except the first mark in the fourth curve, showing approximately the same time intervals from wavelet to wavelet and from the wavelets to the radial. These intervals follow with about the same regularity in selecting different cycles from the same subject, but they differ more in comparing different subjects. There seems to be a time interval of about two and a half or three small squares, that is, 0.021 to 0.025 seconds, between consecutive small wavelets, which is a fairly persistent interval in all the groups. If a wave having these times, 0.021 to 0.025 seconds, as its period, should be sustained long enough to emit a musical tone, it would correspond to a pitch of from forty-eight to forty waves per second, and represent a very low note in the base.

GENERAL FORM OF APEX TRACINGS.

It is evident from an inspection of the apex tracings given in text-figure 1, that there is a great diversity of form and of amplitude

of the apex motion in the different subjects. The same tambour was used for all these tracings. It was round, with a diameter of one and thirteen sixteenths inches. A smaller tambour was made with a diameter of nine sixteenths inches, and the tracing obtained with this was of the same general shape as those given by the larger



TEXT-FIG. 4. The pair of curves, No. 10, are simultaneous tracings from the apex of the heart and the right radial artery with a film moving at 10.85 inches per second, plotted on the same vertical scale as the curves in text-figure 1. Vertical lines mark one tenth second intervals. The pair, No. 17, are simultaneous tracings from the right femoral artery and right radial artery, the film moving at six inches per second. Note the sudden reversal in the femoral at K after the upstroke had begun. This reversal has not been obtained with a normal heart.

one when the smaller tambour was placed with its center upon the same spot as the center of the larger one, but the amplitude of the curve was, of course, less. If either tambour is placed upon a different site, different curves are obtained.

The curves selected for text-figure 1 are chosen to show the great

diversity of apex records rather than their similarity. Curves 2, 5, and 27 are probably records of normal hearts. All the curves except No. 2 are plotted on the scale of one band per half inch, but No. 2 has two bands per half inch. The amplitude of the excursion in curves 5 and 27 is about that which is obtained from the normal subject. Contrast these with the records 29 or 35, which are to the same scale as No. 5. Note the exactness with which the high peaks, in all the apex curves except No. 34, just between the vertical line RR and the A-wave-groups to the left of it, coincide with each other in time; the summit of the wave is very nearly four hundredths of a second prior to the line RR. The peculiarity of the apex curve 34, where the summit of this wave is missing, is noticeable, and it is interesting to see that the radial curve exhibits two very pronounced waves following the highest point after the upstroke. These waves were plainly seen by direct observation of the bands in the instrument before the photograph was taken.

There are no doubt many things which may be revealed by a closer study of apex tracings, but the features which seem the most striking because of their regular occurrence are the A- and B-wave-groups above described.

In conclusion, four tracings are given in text-figure 4, No. 10 being the simultaneous apex and right radial, and No. 17 being the simultaneous right femoral and right radial of the same subject, although taken at a different time from No. 10, so that the pulse rates differ in Nos. 10 and 17. The speed of the film also differed in the two cases; the vertical lines in both the upper and lower part of the figure were spaced one tenth of a second apart, the upper spacing applying to No. 10 and the lower to No. 17. This figure is reproduced on the same scale as text-figure 1, and the great excursion in the case of both the apex and the radial curves is very marked. The radial has more than twice the normal amplitude. The A-wave-group one tenth of a second prior to the line RR is well marked, and immediately following it appears a wave, H, recurring in every cycle.

The femoral tracing was more difficult to obtain than that of a normal subject would have been, because of the sudden reversal of the curve at K immediately after the beginning of the upstroke.

This effect has not been noticed in a normal subject. At the point of reversal, the force was so strong that the bands moved at a rate requiring a higher speed of film than six inches per second, which was the speed used for most of the records. Undoubtedly all of these peculiarities which recur with such regularity in every cycle have a physiological meaning which should be studied, but it is not the purpose of this paper to discuss them.

A STUDY OF TRACINGS FROM THE REGION NEAR THE APEX OF THE HEART.*

By ALBERT C. CREHORE, Ph.D.

(*From the Department of Therapeutics of Cornell University Medical College, New York.*)

In taking tracings from the apex of the heart with the micrograph, the difference in the results obtained by locating the tambour in slightly different sites on the same subject is so great that we have been led to make a chart of the region near the apex showing the exact form of the tracings at definite localities.

The tracings reproduced here were taken from one subject, and a special form of tambour was made to fit between the ribs in the fifth intercostal space, because it was found that the ordinary tambour, which had been used for most apex tracings heretofore, was unsuitable. The old tambour was round, having a diameter of one and thirteen sixteenths inches, and there was so little flesh on this subject that in bridging over the fifth interspace from rib to rib, an open space was left, in the center of the rubber diaphragm covering the tambour, out of contact with that portion of the subject between the ribs which is most effective in transmitting the motion of the heart.

The new form of tambour used for these tracings is shown in text-figure 1. It is made from a block of hard rubber having a cavity milled out to the shape shown, the width of the block being three fourths of an inch, and the width of the opening about a half inch, the length over all being two and three fourths inches and the length of opening two and one sixteenth inches. The opening was covered as usual with a thin sheet of rubber.

The positions of the opening of this tambour directly over the fifth intercostal space are shown with reference to the mid-line of the subject in the diagram, text-figure 2, the five positions from

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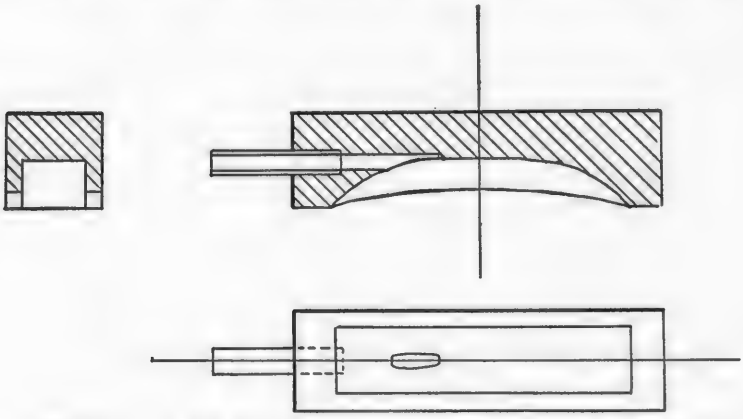
which tracings were taken being numbered consecutively from one to five.

Five tracings from the apex region numbered from the top downward are shown in text-figure 3, each being obtained with the tambour located as indicated by the corresponding number in text-figure 2. Besides these there is one, JJ, taken from the right jugular vein. Records from the right radial pulse in each instance were taken simultaneously; but these are not drawn in full, the beginning of the upstroke of the radial pulse being recorded by a single vertical



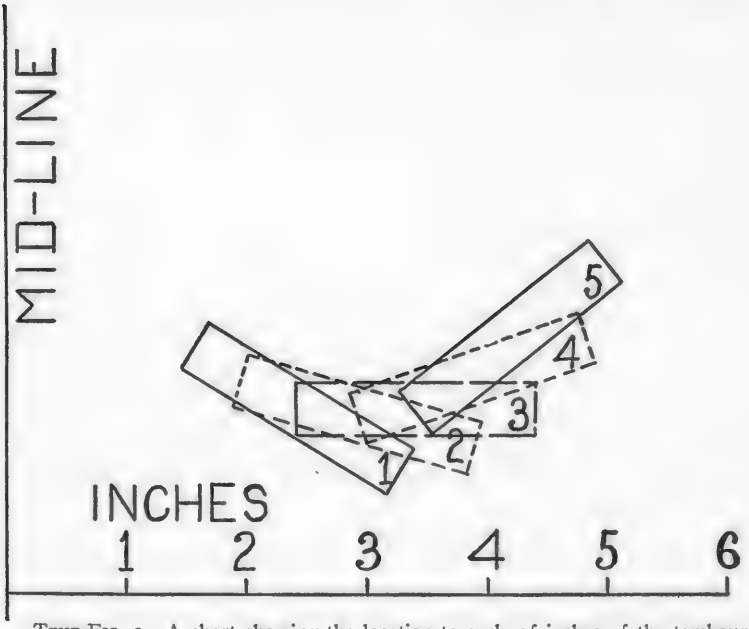
TEXT-FIG. 1. A new form of apex tambour used in obtaining the tracings in text-figure 3.

line only. The vertical line, RR, represents the time of beginning of the upstroke of the radial for all curves.

As the six tracings were not taken at the same time, but on different days within a period of three days, the rates of the pulse and the condition of the subject differ in the various curves. Instead of redrawing each curve to a different scale, we give the curves as recorded, the scale of inches being at the top of the chart. The speed of the moving film was approximately the same in each—8.68 inches per second—and the vertical lines are spaced one tenth of a second apart, taking the beginning of the upstroke of the radial pulse, RR, as a reference line at the one second mark. Since the beginning of the upstroke of the radial in each case is

made to coincide at RR, the next upstroke following will occur at different times according to the rate of the pulse. The short vertical lines, R_1, R_2, R_3, R_4, R_5, show these times for each curve respectively, indicating that No. 5 is the most rapid pulse, sixty-nine per minute, and "J" the slowest, fifty-eight per minute.

An inspection of the curves as a whole shows that the various events are reproduced in their correct time relations, but with some

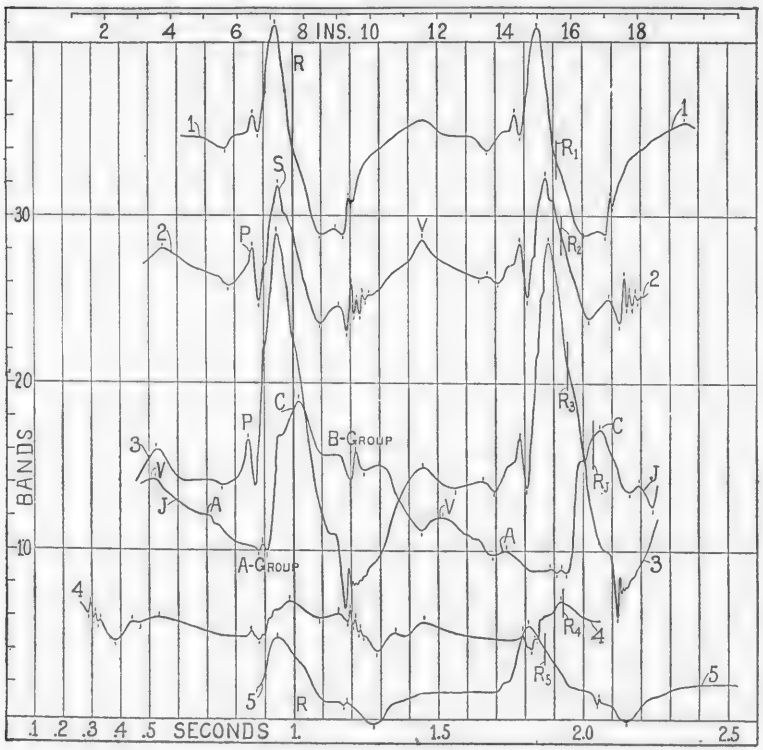


TEXT-FIG. 2. A chart showing the location to scale of inches of the tambour shown in text-figure 1 over the fifth intercostal space, the positions from one to five giving the tracings so numbered in text-figure 3.

variation in the amplitude. One tenth of a second prior to the upstroke of the radial, RR, the A-wave-group[1] appears. Some of the small wavelets in the latter part of this group occur on the steep upstroke of the apex curve before the high peak, S, just preceding the line RR, and are seen as jogs or slight deviations. In the fourth curve, where this peak is low, the wavelets are clearly seen.

[1] *Jour. Exper. Med.*, 1911, xiv, 339.

The B-wave-group two tenths of a second after the line RR appears very prominently in this subject, positions 2, 3, and 4 showing them particularly well. The suggestion of a sudden initial impulse



TEXT-FIG. 3. A series of tracings from the region near the apex of the heart numbered from one to five to correspond with the locations of the tambour in text-figure 2. The time of the beginning of the upstroke of the right radial tracing is marked by the line RR, and the next following upstroke occurs at different times, R_1, R_2, R_3, etc., because the pulse rates differ and the speed of the film is constant; namely, 8.68 inches per second. JJ shows a jugular tracing taken simultaneously with the radial. This shows the well known A, C, and V waves, and in addition the A- and B-wave-groups, one tenth seconds before and two tenths after the line RR respectively.

starting a vibration which is rapidly damped and dies away after a few vibrations is very strong in these three curves; the period

and frequency of the vibration are readily measured. The period is about 0.02 seconds and the frequency about fifty per second, which is in agreement with the pitch of the note elsewhere obtained.[2]

The highest peak, S, occupies nearly the whole time between the A- and B-wave-groups, three tenths of a second, and represents the ventricular systole. Immediately preceding this large S-wave is a smaller wave, P, which appears most pronounced in the second and third tracings. This wave occupies less than a tenth of a second, perhaps something between 0.05 and 0.1 seconds, and coincides in time with the so-called presphygmic period. This is further shown by comparing it with the jugular record, as it follows the well known A-wave caused by the auricular systole.

The Jugular.—Since the tracing, J, from the right jugular vein, was taken synchronously with the right radial and, therefore, is located synchronously with the apex curves, it shows the well known A-, C-, and V-waves of the jugular vein very distinctly, and in addition records the A- and B-wave-groups.[3] These are marked A-group and B-group respectively, and it is observed that they are almost in the same vertical line—one tenth of a second before and two tenths of a second after the line RR,—with the corresponding groups respectively in the five apex curves. The C-wave due to the carotid artery is the highest or most prominent wave in the jugular record and is located almost synchronously with, but a little later than the systolic S-wave in the apex tracings.

The A-wave of the jugular supposed to be due to the auricular contraction appears at A and lasts about one tenth of a second. It precedes the wave, P, in the apex records which we have ascribed to the presphygmic period.

The well known V-wave of the jugular is seen at V in the interval of time between the B-wave-group at the end of systole and the A-wave of auricular contraction. This wave has been ascribed by some writers to the opening of the bicuspid and tricuspid valves admitting blood to the ventricles and relieving the pressure in the vein. Strangely enough the apex tracings show a

[2] *Loc. cit.*
[3] *Loc. cit.*

prominent wave which we have marked V, corresponding in time with that of the jugular.

In conclusion, it is submitted that it is hardly possible for the student who observes the amount of detail shown in these tracings not to be satisfied that the micrograph will be of use not only for research work but also for clinical purposes.

ON THE DISTRIBUTION AND DIRECTION OF MOTION OF THE INTERFERENCE BANDS OF LIGHT FORMED BY THIN PLATES AS THE THICKNESS OF THE PLATE VARIES.*

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Plate 33.

When taking records with the micrograph[1] it was observed that in some instruments the direction of motion of the interference bands of light for an increase of air pressure was opposite to the direction of motion in other instruments. In some the diameters of the circular bands contracted as the two interference plates approached each other, while in others they expanded. Ordinary spectacle lenses were used, and the facts are explained by supposing that the surfaces were curved instead of being plane. It will be shown that there is a critical value of the curvature of the interference surfaces, and when the curvature is greater than the critical value, the bands move in one direction, but when less, they move in the opposite direction.

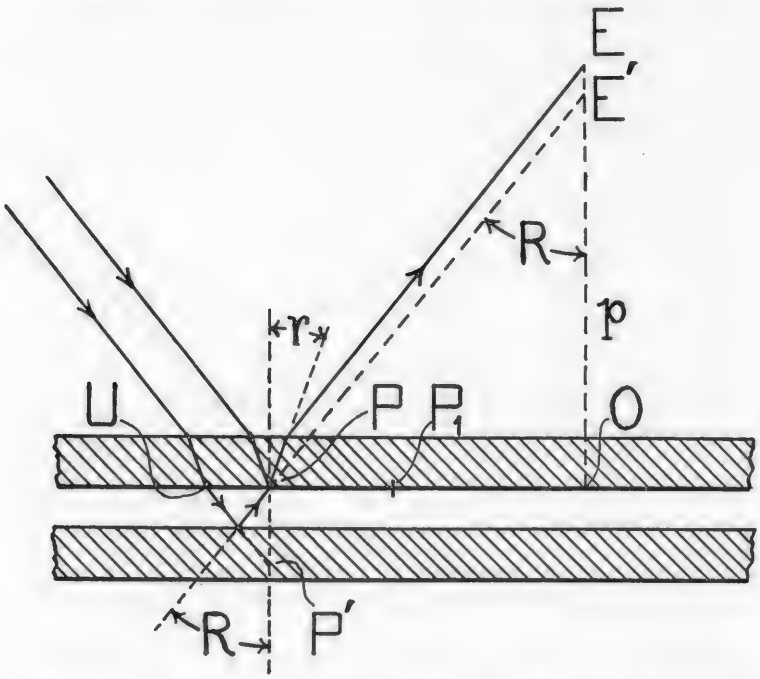
A photograph of interference bands formed between two pieces of plate glass (eight by ten inches), the upper resting upon the lower by its own weight, is reproduced in figure 1, and shows two characteristic kinds of areas that usually appear when two pieces of common glass are placed together. The first kind of area, A, is surrounded by bands in closed curves, which often approximate an elliptical shape. The second kind of area, B, is characterized

* Received for publication, July 10, 1911.

[1] The Micrograph. An Instrument Which Records the Microscopic Movements of a Diaphragm by Means of Light Interference and Some Records of Physiological Events Showing the Registration of Sound Waves Including the Human Voice, Crehore and Meara, *Jour. Exper. Med.*, 1911, xiii, 616.

by there being no bands surrounding it, each band radiating off indefinitely, and by the system of bands resembling pairs of conjugate hyperbolae.

Each of these two classes of areas has another distinguishing feature which is not manifest when the plates are at rest. As the two plates are pressed more firmly together the whole system



TEXT-FIG. 1. Diagrammatical representation of two accurately parallel glass plates showing the path of light from the source to the eye at E.

of bands moves, and those surrounding the area, A, may contract or they may expand as the plates approach each other. If they contract, we may call the curvature of the plates at this point positive, and if they expand, negative. Assuming the area A to be positive, it is evident from the photograph that the hyperbolic bands on the right and left of the area B recede from each other,

while the pair above and below approach each other. The reverse would be true at B if the area A had a negative curvature.

Consider the case of two plates of glass which are exactly parallel, as in text-figure 1. The plates may be imagined to be infinite in extent, and the source of illumination to be pure monochromatic light uniformly distributed over the whole upper hemisphere. Let the observer's eye be located at E, a distance, p, above the interference surfaces. The observer looking at any point, P, of these surfaces sees light by reflection from one point of the source only. Whether or not there is destructive interference at this point P may be determined by the formula:

$$\cos R = \frac{n\lambda}{2\mu e}. \tag{1}$$

R = angle of refraction = $PP'U = PE'O$.
n = any integer.
λ = wave-length of light.
μ = refractive index of the material of the plate.
e = thickness of the air between the two glass plates.

If the equation is satisfied at the point P, there will be total interference, and a dark band will appear at this part of the plates. From symmetry the same values obtain at all points on the circumference of a circle with O as center and OP as radius.

As the point P approaches the point O, the angle R decreases and its cosine increases, and since all the quantities in the right hand member of the equation are constant for a given plate and light source, except n, which is any integer, the equation will not be satisfied again as P approaches O until cos R has increased sufficiently to admit a value of n one unit greater than it had at P. The next consecutive band, therefore, corresponding to a value of n one unit greater, appears as a circle with OP_1, less than OP, as radius. The system of bands seen by the observer at E, therefore, consists of a set of concentric circles centered at O, which extend from an infinite distance, where $R = 90°$, cos $R = 0$, and $n = 0$, to the center, O, where $R = 0$, cos $R = 1$, and therefore,

$$n_0 = \frac{2\mu e}{\lambda}. \tag{2}$$

Since the thin plate is in this case supposed to be air, $\mu = 1$, and

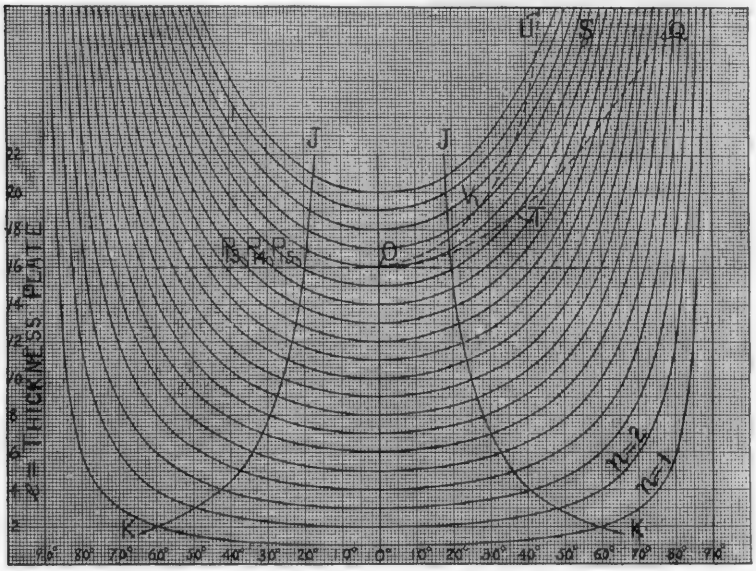
$$n_0 = \frac{2}{\lambda} e. \tag{3}$$

The total number of bands, n_0, constituting the system is, therefore, proportional to the thickness of the plate. When this thickness, e, is equal to one half wave-length of the light, there is but one band in the whole field, and in general the total number of bands composing the system is equal to the number of half wave-lengths of light in the thickness of the plate.

The set of curves shown in text-figure 2 is obtained from equation (1) by putting $\mu = 1$, and writing,

$$R = \cos^{-1}\frac{n\lambda}{2e}. \tag{4}$$

The abscissae give the angles, R, from 0° to 90°, and the ordinates the thickness of plate, e, in half wave-lengths of light for successive values of the integer n, from $n = 0$, to $n = 20$. The set of curves well illustrates the effects produced by separating or approximating the two plates; that is, changing the value of e.



TEXT-FIG. 2. A series of curves representing the required curvature of the upper interference surface, the lower being plane, to produce total interference or darkness over its entire area for any given value of the integer n, the curves being given for all values of n from 0 to 20. The curves JK indicate the diameters of the circles of the first ring, counting from the center outward just as the central ring is appearing or disappearing.

If a horizontal line is drawn at a fixed height, say where $e = 16$ half wave-lengths, this line is tangent to the sixteenth curve at O, and cuts the lower curves successively at the points P_{15}, P_{14}, P_{13}, etc., indicating that with a plate of thickness sixteen, the sixteenth band is just appearing at the center, O, when the fifteenth, fourteenth, thirteenth, etc. bands have radii equal to OP_{15}, OP_{14}, OP_{13}, etc., or about 21°, 28.5°, etc. If the upper plate is raised and the lower remains stationary, the effect on the bands is seen by raising the horizontal line. It

becomes tangent to and then cuts successively the curves corresponding to increasing values of n, and the two points of intersection of this line with any given curve recede from each other, indicating expanding bands, or, *vice versa*, contracting bands, for an approximation of the parallel plates. Parallel plates would, therefore, give a positive instead of a negative area at A, figure 1.

Suppose the lower plate remains plane, and the upper becomes curved—convex toward the plane plate—and is raised at the same time and made to coincide in its upward motion successively with the curves in text-figure 2; in such a case the whole surface would become dark each time a curve is passed in its upward course, because every point of this surface satisfies equation (4) for some value of n.

Imagine a plane surface, say at the height $e = 16$, to be gradually bent from its plane, as OQ, until it comes into coincidence with the curve OS. Evidently the sixteenth band at the common point of tangency, O, will remain undisturbed, and the fifteenth band will recede or expand as the point T advances until it passes entirely off the plate. If the bending of the surface be continued beyond the curve OS to some position as OU, then the seventeenth band appears at an infinite distance and advances to the point V. This curve, OU, shows at a glance that the point V approaches O as the plate is separated more and more from the plane plate below; that is, the whole system of circular bands in this case contracts with a separation of, and expands with an approach of the plates. We have called such a system of bands negative when referring to figure 1.

On the other hand, before the plate is bent sufficiently to coincide with the curve OS, as at OQ for example, it is evident that the point T recedes as the plate is raised, showing expanding bands for a separation of the plates. This corresponds to a positive curvature, as above defined.

The curve OS has, therefore, a critical radius of curvature because the direction of motion of the bands for approaching plates changes sign from contraction to expansion. To find the radius of curvature at the center, O, of the upper plate, equation (4) may be expressed in terms of the radii, $OP = x$, along the plate, and the length of the perpendicular, p, from the eye to the plate, thus,

$$\frac{4}{n^2\lambda^2}e^2 - \frac{1}{p^2}x^2 = 1. \qquad (5)$$

In the following, the thickness of the upper glass plate is considered to be small as compared to the distance, p, from the eye to the plate, so that p is practically constant for all positions of the point P. Considering e and x to be the variables and all other quantities constant, equation (5) represents an hyperbola with semi-major axis $a = n\lambda/2$, and semi-minor axis $b = p$. Since the radius of curvature at the apex of an hyperbola, corresponding to the point O, is $R = b^2/a$, we have

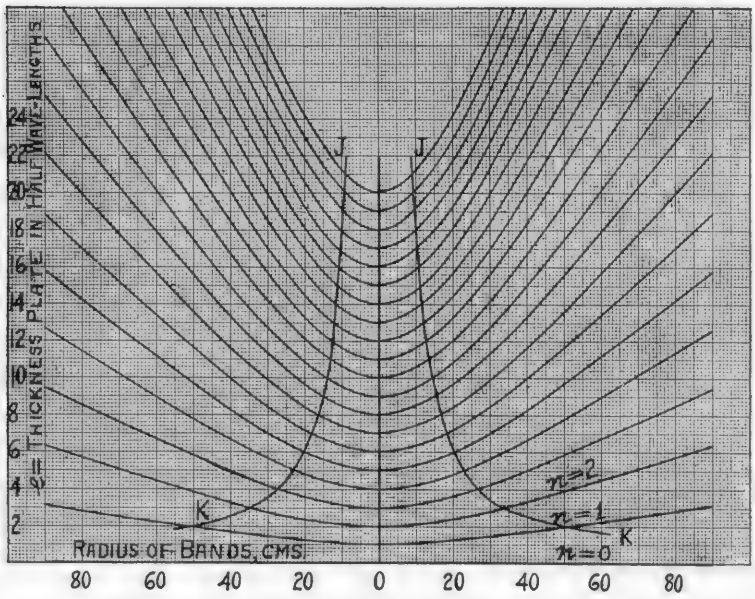
$$R = \frac{2p^2}{n\lambda}. \qquad (6)$$

The set of curves in text-figure 3 is obtained from (5) by assuming the distance from the eye to the plate, p, as 30 cms. One hyperbola is drawn for each value

of n from 0 to 20 as in text-figure 2. The curve JK gives the radius in centimeters of the first band when counting in the reverse order from the center outward, and shows that with parallel plates the diameter of the first band diminishes as the plates separate. Substituting $p = 30$ cms., the radius of curvature in (6) becomes,

$$R = \frac{1800}{n\lambda}.$$

Assuming that the wave-length of the particular light used is 40.47×10^{-6} cms.,



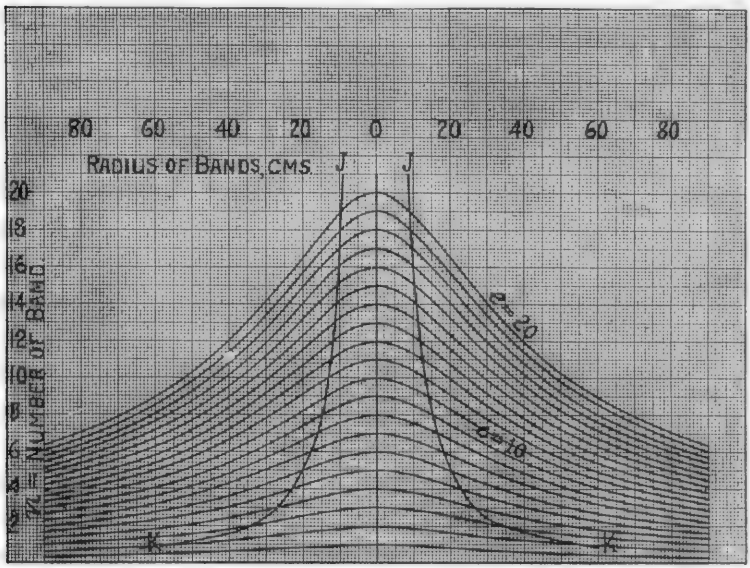
TEXT-FIG. 3. This set of curves is similar to that in text-figure 2 except that the distance p from the eye to the plate (see text-figure 1) is assumed to be 30 cms., and the abscissae are given in centimeters instead of in terms of the angle R in degrees.

we have the following critical radii of curvature for the corresponding values of n:

n	R
1	445. kilometers
10	44.5 kilometers
100	4.45 kilometers
1000	0.445 kilometers

Since by (4), at the origin of the curves where $x=0$, we have $e_0=n\frac{\lambda}{2}$, the values of n above signify the number of half wave-lengths' thickness of the plate at the center, O. In the last case where $n=1000$, the separation of the plates is about four tenths of a millimeter when the critical radius of curvature is nearly half a kilometer.

The same equation (5) may be expressed in a different way, giving the set of curves in text-figure 4 by considering the thickness of the plate, e, to remain con-



TEXT-FIG. 4. A series of twenty curves, each showing the law of increase in the radius of successive circular interference bands for a given thickness of plate e; the several curves corresponding to plates from 0 to 20 half wave-lengths of light in thickness.

stant while the variables are x and n. The transformed equation may be written,

$$x=\rho\sqrt{\frac{4e^2}{\lambda^2}\frac{1}{n^2}-1} \qquad (7)$$

and represents an equation of the fourth degree for these variables.

In general, when two plates of glass are superposed to produce bands as in figure 1, there appear two kinds of critical points or areas, the elliptical and the hyperbolic, as at A and B respectively. The elliptical kind may be either positive or negative, and the surface for either positive or negative has a

definite radius of curvature. The case when the two surfaces are concave to each other and the center is a point of maximum separation would be a case of positive area; when the surfaces are more convex to each other than the critical value, the center is a point of minimum separation and the area is negative.

The second class of critical area, *B*, differs from *A* in there being neither a maximum nor a minimum point, but a critical point of flexure. The two lines taking the direction of the asymptotes to the hyperbolae are approximately parallel with the lower plate, and the curvature of the surfaces between the two asymptotes on opposite sides of the center is of the same kind, both being either positive or negative. The curvature on adjacent sides of one asymptote is opposite, being positive on one side and negative on the other. The asymptotes are themselves the loci of points having a maximum gradient, the surface angle with the parallel plate being here a maximum.

TWO SOURCES OF LIGHT.

There is another interesting effect seen in the photograph, figure 1. The bands P_1, P_2, P_3 appear not to be focused as sharply as the other bands. These blurred bands occur at regular intervals of thirteen bands apart. The explanation is that the picture was not taken with pure monochromatic light, but with a mercury vapor lamp without the use of screens. The spectrum of this light has four prominent bright lines, one in yellow, green, blue, and violet, respectively.

The wave-lengths of the two latter colors are: blue, 43.59×10^{-6} cms., and violet, 40.47×10^{-6} cms. Commercial Seed's plates were used, and they were not sensitized for the yellow and green lines of the spectrum. The picture shows, however, that both of the lines of shorter wave-length affected the plate, one more than the other.

If two wave-lengths, λ_1 and λ_2, are employed, and the corresponding numbers of the bands counting from an infinite distance to a point, *P*, of the plate be denoted by n_1 and n_2 respectively, we have from (1),

$$\cos R_1 = \frac{n_1\lambda_1}{2\mu e} \quad \text{and} \quad \cos R_2 = \frac{n_2\lambda_2}{2\mu e},$$

from which we obtain the relation, when $R_1 = R_2$,

$$\frac{n_1}{n_2} = \frac{\lambda_2}{\lambda_1}. \tag{8}$$

In the case of the mercury vapor lamp, using wave-lengths 43.59 and 40.47 cms., we have,

$$\frac{\lambda_2}{\lambda_1} = 1.077 = \frac{14}{13} \text{ approximately.} \tag{9}$$

Hence,

$$\frac{n_1}{n_2} = \frac{14}{13} = \frac{28}{26} = \frac{42}{39} = \text{etc.} \tag{10}$$

The first dark band corresponding to $n = 0$ occurs at an infinite distance for all wave-lengths, but the next point where the dark bands coincide for these

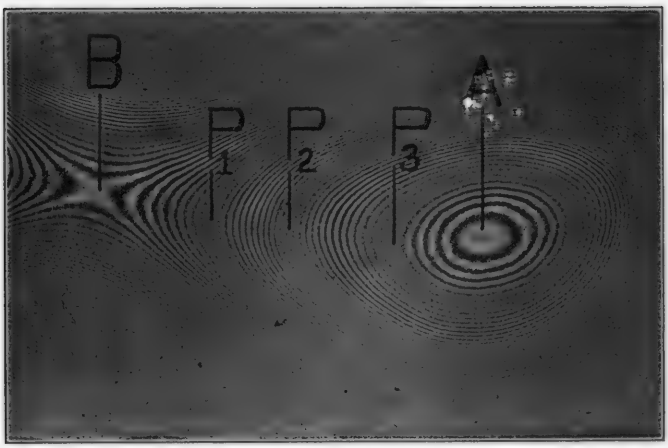


FIG. 1.

particular wave-lengths is thirteen bands for the one and fourteen bands for the other, counting from an infinite distance toward the center. Succeeding coincidences will occur at all multiples of 13 and 14 until the center, *O*, is reached.

At these points of coincidence of the dark bands, the picture should be sharp with strong contrast, but half way between these positions the blurred effect should be most pronounced. If the actinic effect of the two sources upon the plate is very unequal, the blurred region should be confined to a smaller area than it would be were the two sources equally intense. The fact that this effect is confined to one or two bands shows that the two sources in the mercury vapor lamp have an unequal effect upon the plate.

A complete explanation of all the points considered arising from the interference of light between two glass plates, as seen in figure 1, has thus been obtained from an application of the very simple formula (1).

EXPLANATION OF PLATE 33.

FIG. 1. A photograph of light interference bands obtained with two pieces of plate glass, eight by ten inches, and a mercury vapor light, the plates being observed by reflected light. The areas A and B are each typical of those observed when two common pieces of glass are used. Note that the bands appear indistinct at regular intervals of thirteen bands P_1, P_2, P_3. This is explained by the character of the light and the kind of photographic plate used.

EXPERIMENTAL ACUTE NEPHRITIS: THE ELIMINATION OF NITROGEN AND CHLORIDES AS COMPARED WITH THAT OF PHENOLSULPHONEPHTHALEIN.*

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AND

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This investigation was undertaken, at the suggestion of Dr. Pearce, as complementary to the studies of one of us,[1] on the elimination of phenolsulphonephthalein in a variety of experimental renal lesions. Its object has been to determine whether or not the elimination of this dye parallels that of the chlorides and the total nitrogen. The determination of this point is of importance, not only for the interpretation of the clinical value of the phenolsulphonephthalein test, but also as a means of adding to our knowledge of the normal physiology of the kidney.

Methods.—The dog has been used in all the experiments. Several forms of experimental nephritis were selected for study; namely, those due to uranium nitrate or to potassium chromate, representatives of the so-called tubular (or epithelial) nephritis type; and that caused by cantharidin, usually considered to be a nephritis of the vascular type. The various poisons have been given in small doses, since the use of large doses has invariably resulted in vomiting, diarrhea, or loss of appetite, thus rendering the nitrogen and chloride estimations relatively valueless. With small doses, the

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, July 11, 1911.

[1] Eisenbrey, A. B., A Study of the Elimination of Phenolsulphonephthalein in Various Experimental Lesions of the Kidney, *Jour. Exper. Med.*, 1911, xiv, No. 5 (November).

lesions caused by cantharidin have not been severe; those due to uranium nitrate and potassium chromate have, however, been marked. All animals were kept in the conventional metabolism cages and, after a preliminary period of observation for the detection of spontaneous lesions, were placed on a constant diet. This consisted of beef heart, casein, lard, and crackers, in such proportions that, when in nitrogen equilibrium, the daily elimination of nitrogen in the urine approximated one gram per kilo of body weight. The object was thus to bring about, without causing digestive disturbances or loss of appetite, as high a daily nitrogen elimination as possible. The importance of such a high nitrogen elimination will be discussed in connection with the experiments with uranium. To this diet was added, daily, one gram of sodium chloride and one gram of bone ash, and a measured quantity of water. The nitrogen and chloride estimations were made each day on the total twenty-four hour urine from catheterized animals. On the days when the phenolsulphonephthalein elimination was determined, two sets of estimations were made: one on a composite sample from the urine collected during the two hours required for the phenolsulphonephthalein test, the other from the urine collected during the remaining twenty-two hours. The Kjeldahl method was used for the determination of nitrogen, and the modified Volhard method for chlorides. In making the nitrogen determinations, coagulable protein was first removed by boiling and acidulation with acetic acid. Control estimations to determine the effect of the administration of phenolsulphonephthalein upon the nitrogen or chloride elimination showed no alteration in either. The elimination of phenolsulphonephthalein was determined by the original method of Rowntree and Geraghty.[2]

Experiments with Uranium Nitrate.—Uranium nitrate was used in 0.75 per cent. aqueous solution and injected hypodermically into the flank, as indicated in the tables.

It will be seen that during the two days following the administration of uranium nitrate a fall in the nitrogen occurred, comparable

[2] Rowntree, L. G., and Geraghty, J. T., An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, *Jour. Pharmacol. and Exper. Therapeutics,* 1909, i, 579.

TABLE I.

Experiment I. Dog 19, weighing 10,420 gms., was placed, on May 28, on a constant diet of beef heart, casein, cracker, and lard, containing 10.58 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 500 c.c. of water. The urine contained no albumin or casts from this time until June 7.

Date.	Weight in gms.	Phenol-sulph. test, 10 A. M.	Uranium nitrate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein Esbach.	Urinary data refer to twenty-four hour specimens beginning 9 A. M. on date specified.
June 5	10,370			490	7.90	2.00	0	
" 6	10,430	69.0 %		500	8.07	2.12	0	
" 7	10,465		3 P.M. 0.015 gm.	520	6.87	1.88	Trace	Few fine granular casts.
" 8	10,430	72.8 %	Noon 0.01 gm.	478	5.19	1.37	0.6 gm.	Few fine granular casts.
" 9	10,225	56.6 %						Slight vomiting.
" 10	9,940	14.0 %						Vomiting. Chloroformed.

On microscopic examination of the kidneys, the glomeruli appeared normal. The epithelium of the convoluted tubules showed "granular" degeneration and fat vacuoles; the nuclei were pale. The medullary loops contained granular casts. The vessels were not congested.

to that noted by Pearce, Hill, and Eisenbrey[3] and by Siegel,[4] in the dog, and by Schlayer and Takayasu[5] in the rabbit. The vomiting during the third day rendered further analyses worthless. The chlorides of the urine showed a distinct drop; this also was noted by the above mentioned investigators. The fall in the phenolsulphonephthalein elimination was less prompt than the fall in the nitrogen and chlorides, but eventually more marked. Synchronously with this fall in the phenolsulphonephthalein elimination, the condition of the dog became poor, and vomiting and loss of appetite set in. During the experiment, the animal was placed on a diet containing somewhat more than one gram of nitrogen per kilogram of body weight and showed, in consequence, a high nitrogen elimination during the control period. A comparison with experiment

[3] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., Experimental Acute Nephritis: The Vascular Reactions and the Elimination of Nitrogen. *Jour. Exper. Med.*, 1910, xii, 196.

[4] Siegel, W., Ein Stoffwechselversuch bei Urannephritis am Hunde, *Ztschr. f. exper. Path. u. Therap.*, 1907, iv, 561.

[5] Schlayer and Takayasu, Untersuchungen über die Funktion kranker Nieren., *Deutsch. Arch. f. klin. Med.*, 1910, xcviii, 17.

II shows that such a high nitrogen diet is a necessary condition for the study of the power of the kidney to eliminate nitrogen.

TABLE II.

Experiment II. Dog 1, weighing 8,415 gms., was placed, on March 14, on a constant diet of beef heart, casein, cracker, and lard, containing 3.61 gms. nitrogen, with 1 gm. bone ash and 350 c.c. water. The urine from that date until March 21 was free from albumin and casts.

Date.	Phenol-sulph. test, 10 A. M.	Uranium nitrate.	Urine.		Notes.
			Amount in c.c.	Total nitrogen in gms.	Urinary data refer to twenty-four hour specimens beginning 9 A. M. on date specified.
				2.94	No albumin or casts.
Mar. 19	77.8 %		228	3.28	No albumin or casts.
" 21		11 A.M. 0.015 gm.		3.04	Albumin 1/6 by volume; few fine granular casts.
" 22	25.3 %		415	3.39	Albumin 1/6 by volume; few fine granular casts.
" 23	4.1 %		485	3.37	Albumin 1/6 by volume; few fine granular casts. Very slight vomiting.
" 24	3.51 %				Vomiting marked.
" 25	1.8 %				Vomiting marked.
" 26	1.55 %				Vomiting marked. Chloroformed.

Microscopic examination of the kidney showed congestion of the glomeruli and intertubular spaces of both cortex and medulla and the presence of subcapsular and interstitial hemorrhages. The cells of the convoluted tubules were swollen and turbid and the lumina of these and of the medullary loops contained hyaline and fine granular casts.

In this experiment, the diet was much lower in nitrogen than in experiment I, although the amount of nitrogen was still adequate for the requirements of the animals, and no fall in the elimination of nitrogen occurred. The dog in experiment I, even at the time when its nitrogen elimination was impaired, excreted 5.19 grams; the dog in experiment II, on the other hand, because of the low nitrogen diet, had never more than 3.39 grams of nitrogen to excrete and continued to maintain the nitrogen elimination undiminished in spite of renal injury. The impairment which doubtless did exist in the excretory power of the kidney remained undemonstrated. In this instance it is evident that the nitrogen retention was more readily determined in an animal on a high nitrogen equilibrium than in one on a low equilibrium. In all the other experiments reported, we employed, therefore, a high nitrogen diet.

The phenolsulphonephthalein test in experiment II showed a somewhat more rapid fall than in experiment I. It is of interest that on the day when the elimination had fallen to the very low figure of 4.1 per cent., the kidney still eliminated readily 3.37 grams of nitrogen and an increased quantity of water. It is also of interest that vomiting began, as in experiment I, when the functional test had reached a low figure. This fact will be referred to later.

In experiment III, the attempt was made to produce a nephritis with less gastric disturbance, and for this purpose only half the previous dose of uranium was employed.

TABLE III.

Experiment III. Dog 17, weighing 7,450 gms., was placed, on May 16, on a constant diet of beef heart, casein, cracker, and lard, containing 7.32 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 300 c.c. of water. The urine contained no albumin or casts from this time until May 23.

Date.	Weight in gms.	Phenol-sulph. test, 10 A. M.	Uranium nitrate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	Urinary data refer to twenty-four hour specimens beginning 9 A. M. on date specified.
May 20	7,450	79.5 %		210	5.75	1.53	0	
" 22	7,450	78.0 %		285	5.79	1.71	0	
" 23	7,360		2 P.M. 0.0075gm.	291	5.91	1.99	0	
				250	6.27	1.77	Faint trace	Few pale granular casts.
" 24	7,350	78.8 %		404	6.78	2.20	Light cloud	Many pale granular casts.
" 25	7,230	68.6 %		536	7.32	1.31	0.5gm.	Many pale granular casts.
" 26	7,050	44.4 %						Vomiting.
" 27	6,720	12.8 %		646	7.06	0.73	0.7gm.	Very little vomiting.
" 28	6,440							Chloroformed.

Microscopic examination showed the glomeruli and interstitial tissue of the cortex and medulla to be moderately congested. The cells of the convoluted tubules showed cloudy swelling. Their nuclei were well stained; the lumina contained granular debris. The medulary loops were filled with hyaline and granular casts.

The experiment of using the smaller dose of uranium nitrate was repeated three times and the same rise in the nitrogen elimination always occurred. The explanation of this increased nitrogen excretion after a small dose is uncertain, but, in view of the dis-

tinct loss of body weight, it would appear that an increased katabolism is caused by uranium, while at the same time the eliminative function of the kidney is not sufficiently impaired by the small dose to interfere with the excretion of the excess of waste nitrogen. An early increase in the elimination of chlorides associated with diuresis occurred, followed by a reduced chloride elimination. Whether this fall is to be considered as compensatory for the earlier excessive elimination, or as a retention from diminished excretory power is a question. The delay in the elimination of the phenolsulphonephthalein was quite similar to that observed with the larger doses of uranium, and a very low figure was reached at a time when the nitrogen elimination was still above normal.

Experiments with Potassium Chromate.—A 3 per cent. aqueous solution of potassium chromate was injected hypodermically as shown in the tables. The dose employed was the largest that could be given without encountering the risk of early vomiting.

Microscopic examination showed the glomeruli to be normal. The tubular epithelium was the seat of fatty degeneration and the nuclei were large and pale. The lumina contained granular debris. A moderate interstitial edema was present in the medulla.

In this experiment a transient fall in the nitrogen occurred after each injection, within either twenty-four or forty-eight hours. The chlorides showed a similar fall, followed by a compensatory rise. Pearce, Hill, and Eisenbrey noted a similar fluctuation in the nitrogen elimination of the dog after chromate; and Green,[6] following the use of a very large dose of the poison, obtained in the rabbit a similar though somewhat more prolonged fall in both nitrogen and chlorides during the first three days. The delay in the phenolsulphonephthalein elimination developed more slowly and was less marked than in the experiments with uranium. It will be noted that some of the lowest figures for the functional test were synchronous with a high nitrogen and chloride elimination. Vomiting began, as with uranium nephritis, when the functional test had reached a low figure.

[6] Green, J. L., Experimental and Clinical Observations on the Urine and Blood in Nephritis, *Jour. Path. and Bact.*, 1909, xiii, 296.

TABLE IV.

Experiment IV. Dog 4, weighing 6,370 gms., was placed, on April 10, on a constant diet of beef heart, casein, cracker, and lard, containing 7.18 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 300 c.c. of water. The urine was free from albumin and casts from this date until April 14.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Potassium chromate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	The urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
April 12	6,370	72.0 %		245	6.51		0	
" 13	6,275			233	7.01		0	
" 14	6,270		Noon 0.03 gm.	237	6.88		0	
				275	5.82		0.1 gm.	Few fine granular casts.
" 15	6,300	66.2 %		413	6.26		0.6 gm.	Few fine granular casts.
" 16		63.0 %		446	6.46		0.3 gm.	Few fine granular casts.
" 17	6,000	65.2 %					Cloud	
" 18	6,040	63.8 %	Noon 0.03 gm.				Cloud	
								Error in diet. Intermission.
May 3	5,570						Trace	Constant diet resumed.
" 5	5,670			320	6.26		Faint trace	Occasional granular casts.
" 6	5,535			320	5.98		Trace	Occasional granular casts.
" 7	5,650			330	6.03	1.52	Trace	Occasional granular casts.
" 8	5,650	66.4 %		330	6.56	1.75	Faint trace	Occasional granular casts.
" 9	5,660	72.2 %	2 P.M. 0.03 gm.	355	6.40	1.75	Light cloud	Many granular casts.
" 10	5,645	58.2 %		378	5.89	0.97	0.7 gm.	Many granular casts.
" 11	5,645	46.3 %		380	6.95	1.96	0.1 gm.	Many granular casts.
" 12	5,660	59.3 %	Noon 0.03 gm.	300	6.08	1.58	0.6 gm.	Many granular casts.
" 13	5,720	51.2 %		340	6.77	1.77	0.6 gm.	Many granular casts.
" 14		45.6 %	2 P.M. 0.03 gm.	426	6.92	2.15	0.5 gm.	Many granular asts.
" 15	5,630	45.6 %						Trace of vomitus.
" 16	5,570	42.5 %						Trace of vomitus.
" 17	5,690							Chloroformed.

In order to secure with this poison a more rapid fall in the phenolsulphonephthalein elimination, two doses of chromate were given on successive days. The result is shown in table V.

Microscopic examination showed the glomeruli to be normal. The tubular epithelium was the seat of marked cloudy swelling, with slight fatty degenera-

tion; the lumina were filled with finely granular debris. A slight interstitial edema was noted in the medulla.

TABLE V.

Experiment V. Dog 20, weighing 5,460 gms., was placed, on May 27, on a constant diet of beef heart, casein, cracker, and lard, containing 6.43 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 350 c.c. of water. The urine was free from albumin and casts from this date until June 11.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Potassium chromate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	The urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
June 8	5,430			350		1.37	0	
" 9	5,430	78.9 %		370	6.17	1.32	0	
" 10	5,470	70.9 %		550	6.11		0	
" 11			Noon 0.03 gm.	380	5.52	1.27	Faint trace	Few granular casts.
" 12	5,450	40.9 %	2 P. M. 0.03 gm.					Vomiting.
" 13	5,465	3.54 %						Chloroformed.

In this experiment, a prompt fall in nitrogen was evident, but it was somewhat less marked than in experiment IV. A drop in chlorides was not observed during the first twenty-four hours. The fall in the phenolsulphonephthalein elimination was, however, more prompt and marked than in dog IV, as was also the vomiting.

Experiments with Cantharidin.—With this drug two kinds of experiments were made, according as a single large dose, or daily small doses were given. The cantharidin was administered hypodermically in the form of a saturated solution in acetic ether.

The first type is illustrated in table VI.

Microscopic examination of the kidneys showed the cortex, medulla, and especially the glomeruli to be intensely congested. The epithelium of the convoluted tubules showed a marked granular degeneration, with entire loss of cellular outline and very pale swollen nuclei. The epithelium of the other tubules was swollen and turbid. The lumina of the convoluted tubules and of the medullary loops were completely occluded by the swelling of the epithelium. The medulla showed moderate interstitial edema.

In this experiment, in the first twenty-four hours, with free diuresis, a fall in the nitrogen elimination occurred. That this fall was not due to the very slight vomiting was proved by the fact that the total

TABLE VI.

Experiment VI. Dog 6, weighing 5,760 gms., was placed, on April 2, on a diet of beef heart, casein, cracker, and lard, containing 6.88 gms. nitrogen, with 1 gm. bone ash and 300 c.c. water on April 2. The urine was free from albumin and casts from this time until April 16.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Cantharidin.	Urine.			Notes.
				Amount in c.c.	Total nitrogen in gms.	Coagulable protein in gms., Esbach.	The urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
April 12	5,715			295	5.36	0	
" 14	5,695			320	5.54		
" 15	5,765	66.0 %		275	5.73		
" 16	5,710		5 P.M. .02 gm.	560	3.24	0.1	Very slight vomiting. Hematuria.
" 17	5,285	9.1 %					Marked vomiting; animal refuses food; anuria. Chloroformed.

nitrogen of urine and vomitus during the twenty-four hours was only 3.74 grams. The fall in the phenolsulphonephthalein elimination was prompt and very marked and coincided with the onset of vomiting.

The second type of experiment is illustrated in table VII.

Microscopic examination of the kidneys showed congestion of the glomeruli and a deposit of precipitated serum between the tuft and the capsule of Bowman. The cortex and medulla were moderately congested. The epithelium of the convoluted tubules was the seat of marked granular degeneration with fatty vacuoles; the nuclei were pale and swollen and the lumina were distended with granular debris. The walls of the medullary tubules were in apposition, the lumina being occluded, and the medulla showed slight interstitial edema.

In this experiment the nitrogen excretion was increased. The loss of weight, conspicuous after small doses of uranium, was very slight, and therefore the interpretation of the increased nitrogen excretion is less clear. The excess of diuresis in a given day bore no relation to the amount of nitrogen eliminated, but was followed, in a general way, at least for a few days, by the chloride elimination. The phenolsulphonephthalein elimination was only very slightly affected and not until after the sixth injection. No vomiting occurred. In spite of marked anatomic alteration in the kidney, the elimination of nitrogen, chlorides, and phenolsulphonephthalein

TABLE VII.

Experiment VII. Dog 16, weighing 10,620 gms. was placed, on May 16, on a constant diet of beef heart, casein, cracker, and lard, containing 10.59 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 400 c.c. water. The urine was free from albumin and casts from this date until May 22.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Cantharidin.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	Urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
May 19				220	8.52	1.52	0	
" 20	10,620	72.1 %		330	8.83	1.72	0	
" 21	10,640	80.7 %		285	8.30	1.56	0	
" 22	10,680		2 P.M. 0.006 gm.	390	9.17	2.69	0	
" 23	10,450	81.0 %		296	10.62	1.91	0	
" 24	10,550	87.5 %	2 P.M. 0.006 gm.	602	9.47	3.06	Trace	Erythrocytes in urine.
" 25	10,420	75.0 %	2 P.M. 0.006 gm.	312	10.58	1.07	Trace	Erythrocytes and few granular casts.
" 26	10,440	81.9 %	2 P.M. 0.006 gm.	492	11.29	2.88	Faint trace	Erythrocytes and few granular casts.
" 27	10,420	79.1 %	2 P.M. 0.006 gm.	509	9.73	1.75	Faint trace	Erythrocytes and few granular casts.
" 28	10,170		2 P.M. 0.006 gm.	420	9.45	0.84	Light cloud	Erythrocytes and few granular casts.
" 29	10,080		2 P.M. 0.013 gm.				Light cloud	Animal refuses part of food; erythrocytes and few granular casts.
" 30	9,975	67.5 %	2 P.M. 0.013 gm.				Light cloud	Animal refuses part of food; erythrocytes and few granular casts.
" 31		65.7 %					Faint trace	Animal eats all of food again; erythrocytes, few granular casts.
June 3		57.8 %		460	7.20	1.32	0	Animal eats all of food again; erythrocytes and few pale granular casts.
" 4		78.8 %		445	8.82		Faint trace	Animal eats all of food again; erythrocytes and few pale granular casts.
" 5	9,880							Animal does not eat. Chloroformed.

was excellent, and the only symptom of intoxication observed was slight loss of appetite.

Experiments with arsenious acid were attempted, but were unsatisfactory, in that doses large enough to cause a frank nephritis were followed by immediate vomiting which prevented metabolic

study. Such lesions as were produced did not, however, alter the elimination of phenolsulphonephthalein.

CONCLUSIONS.

1. Uranium and cantharidin, in the smallest doses capable of producing a distinct nephritis, tend to increase the elimination of nitrogen, probably by stimulating tissue katabolism.

2. Uranium, cantharidin, and potassium chromate, in larger doses, impair the power of the kidney to eliminate nitrogen; but this may not be evident unless the animal is on a high nitrogen diet, and the impairment, when due to potassium chromate, may not persist more than a day.

3. Small doses of uranium and of cantharidin cause a transient increase of chloride elimination which corresponds in a general way to the excess of diuresis.

4. Large doses of uranium and of chromate cause a fall, usually transient, in the chloride elimination. The chloride elimination may, however, be diminished forty per cent. for twenty-four hours without evidences of intoxication (vomiting).

5. The anatomic appearance of the kidney varies somewhat with the poison used and greatly with the period of survival after administration of the poison, but bears no definite relation to the nitrogen, chloride, or phenolsulphonephthalein elimination; marked anatomic alteration is compatible with normal elimination of all these substances and with freedom from symptoms of intoxication (vomiting).

6. The decrease in the elimination of phenolsulphonephthalein, which occurs in uranium, chromate, and cantharidin nephritides, and which, in a general way, is proportional to the dose of the poison, bears no constant relation to the changes in the nitrogen or chloride elimination.

7. A marked decrease in the elimination of the phenolsulphonephthalein occurs synchronously, as a rule, with the onset of the symptoms of intoxication (vomiting), and therefore the phenolsulphonephthalein test would seem to be a better indicator of the ability of the kidney to eliminate the toxic substance responsible for the symptoms of renal insufficiency than are either the anatomic changes or the elimination of total nitrogen or of chlorides.

SO-CALLED BIOLOGICAL TESTS FOR ADRENALIN IN BLOOD, WITH SOME OBSERVATIONS ON ARTERIAL HYPERTONUS.*

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PLATES 34–37.

In the absence of chemical methods of sufficient delicacy, various biological methods have been proposed for the detection of adrenalin in the minute amounts in which it can be supposed to exist in the blood. For some of these the claim is made that the pressor principle of the adrenal glands can be not only detected but quantitatively estimated by their aid. Without any risk of appearing censorious, it can, I think, be said that some of these methods have been adopted for clinical investigations without being sufficiently tested by exact physiological experiments and without a just sense of the great care which is necessary in interpreting the results. I was first made aware of this about two years ago in the course of some studies on cases of persistently high arterial blood pressure, particularly in nephritis. The obvious question arose, whether the presence of an abnormally high content of adrenalin in the blood might not be a factor in the maintenance of the high blood pressure. On turning to the literature, I found that the question had apparently just been answered by A. Fraenkel,[1] who stated that the adrenalin content of the blood was not increased in chronic nephritis, although it was markedly augmented in a case of Graves' disease investigated by him. The adrenalin in normal human serum was, according to him, perfectly capable of detection, and its amount varied within the same limits as in the nephritic serum. His conclusions were based

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[1] *Arch. f. exper. Path. u. Pharmakol.*, 1909, lx, 395.

entirely upon a comparison of the behavior of isolated rings of rabbit uterus when immersed in Ringer's solution, in human serum, and in adrenalin solutions respectively. Oxygen was allowed to bubble through the liquids. Sooner or later these uterus preparations in the oxygenated Ringer's solution began to execute fairly regular spontaneous contractions. When a liquid containing adrenalin in minute amount (a concentration as small as one to twenty millions is still capable of detection) was substituted for the Ringer's solution, a change in the curve took place characterized by an increase in tone and in the frequency of the contractions. Normal human serum occasioned an exactly similar change and so did serum from the pathological cases examined. Conclusions as to the amount of adrenalin in such sera were arrived at by fixing the greatest degree of dilution of the sera (with Ringer's solution) at which they were still active. Fraenkel recommends that the rabbits employed should be vigorous adults, not pregnant, or at any rate only at the beginning of pregnancy. It is known that in cats the effect of stimulating the sympathetic and also the action of adrenalin on the uterus depend on whether the uterus is or has ever been pregnant or is virgin.[2] In the virgin cat, adrenalin (or stimulation of the hypogastric) causes inhibition only, which persists long after the blood pressure has returned to normal. In pregnant cats, the effect is contraction pure and simple, and this reaction generally persists after pregnancy. In the rabbit, as a general rule, according to Cushny, pure contraction is caused, sometimes contraction followed by relaxation and inhibition. In one rabbit he thought that a very small dose of adrenalin induced slight inhibition, while a larger injection was followed by simple contractions, and in one pregnant rabbit in which hypogastric stimulation induced inhibition, the same effect followed adrenalin. Cushny is speaking of the effects produced by injection of adrenalin into the circulation with the uterus *in situ*. A certain amount of variability, then, exists in the action of adrenalin even in the case of the rabbit uterus. But its action is always the same as that caused by stimulation of the sympathetic supply, and the variability is of no consequence for the test, if on each preparation the effect of

[2] Cushny, *Jour. Physiol.*, 1906–7, xxxv, 1; Dale, *ibid.*, 1906, xxxiv, 188.

adrenalin is compared with that of the blood or serum under investigation.

Seeing that adrenalin has the most characteristic effect of causing always those reactions in smooth muscle innervated by the sympathetic which would have been caused by stimulation of the sympathetic supply, it was rather surprising to find that it was not considered necessary to test in each case the action of the fluids examined on other tissues, especially on tissues like the intestine, in which adrenalin produces inhibition instead of increase of contraction. For while it seemed very obvious that the contact of a complex liquid, especially of an alien one like human serum with rabbit uterus might cause an increase of tone,—a not uncommon sign of injury in muscular tissues, even if no adrenalin were present,—the probability that the change was really due to adrenalin would be much increased if the characteristic adrenalin reactions were caused in other tissues, particularly in those whose response to adrenalin is the inverse of that of the uterus. Two properly chosen reactions would seem to represent the minimum number on which any conclusion could be based as to the presence or absence of adrenalin in a liquid so complex as blood. Also the quantitative variations in the reactions must agree when the serum is diluted to a definite extent. The use of the uterus method for clinical purposes by the present writer confirmed the attitude of suspicion towards results obtained by its use alone. I found, indeed, that human serum might cause changes in the curve such as were described by Fraenkel[3] (figures 12 and 13) and that very similar changes were caused by adrenalin (figure 14), but my experience of the method only strengthened the conviction, that, while it might possibly be suited to act as one of a pair of test reactions, it could not, standing by itself, prove the presence of adrenalin. Fraenkel himself admits this. A proof he says is only obtained by the agreement of different methods founded on different physiological actions of adrenalin. And he finds corroboration of the conclusion that normal serum contains adrenalin in the results of the Meltzer-Ehr-

[3] *Loc cit*

mann[4] method on the enucleated frog eyeball, and in those of O. B. Meyer[5] on stretched strips of ox artery. But the frog eyeball method has yielded negative results with human serum in the great majority of cases. Thus Comessatti,[6] in twenty-two cases of renal disease which showed normal, subnormal, or increased blood pressure obtained a positive reaction (dilatation and rounding out of the pupil), and that a weak one, in only one case. Meyer's method is apparently unsuitable for testing alien serum, or at least distinctly less suitable than for serum from the same species of animal.[7]

Battelli's statement,[8] that concentrated human serum when injected into the circulation of animals causes a rise of blood pressure similar to that produced by adrenalin, can by no means be accepted without examination. For Schlayer has shown that concentration of serum *in vacuo* does not lead to its enrichment in adrenalin or rather to an increase in the action supposed to prove the presence of that body, but to a diminution which may reach 30 per cent. In five specimens of serum obtained from four persons, I observed, with three specimens, a fall of blood pressure on intravenous injection of the serum into dogs, and no effect with the remaining two (figures 1, 2, 3, and 9). Two of the specimens were from the same person, a man (T.) with a persistently high blood pressure and a considerable amount of albumin and casts in his urine. A specimen obtained on April 26, 1909, caused a fall of blood pressure (figure 2), while a specimen obtained on May 15th, 1909, produced only the same slight rise of pressure as was seen in the control observation with sodium chloride solution (figure 3). A specimen of serum from another patient (C.) with a still higher blood pressure but no albumin in the urine produced a distinct though transient fall of pressure when the vagi were intact (figure 9), but no change in the same animal after the vagi were cut. It is not easy to see in the case of these three specimens how "concentrated" serum would

[4] Meltzer, *Am. Jour. Physiol.*, 1904, xi, 449; *Deutsch. med. Wchnschr.*, 1909, xxxv, 575; Ehrmann, *Arch. f. exper. Path. u. Pharmakol.*, 1905, liii, 97; *Deutsch. med. Wchnschr.*, 1909, xxxv, 676.

[5] *Ztschr. f. Biol.*, 1906, xlviii, 352.

[6] *Arch. f. exper. Path. u. Pharmakol.*, 1909, lx, 233.

[7] Schlayer, *München. med. Wchnschr.*, 1908, lv, 2604.

[8] *Compt. rend. Soc. de biol.*, 1902, liv, 1179.

be likely to reveal an adrenalin action by a rise of blood pressure, even on the supposition that the serum became actually richer in adrenalin by the concentration, since the substances which by hypothesis are masking the adrenalin action must have become concentrated in the same degree. The possibility remains, of course, that in the case of the two specimens which gave a negative result, concentration might have developed a pressor effect. But even then if the adrenalin action is overborne by effects of the serum leading to a fall of pressure in three specimens out of five, it is certainly not a hopeful procedure for the clinical detection of adrenalin to inject human serum or blood whether concentrated or not into the circulation of an animal. The experiment is much too complicated. For the liquid to be tested contains, or may contain, many substances with different or even opposite physiological actions, and the test object is made up of numerous mechanisms, an action on one of which may entail secondary effects on others, which cannot be distinguished from the primary effects of some of the substances in the serum. Simplification must be sought either by "fractionating" the serum so as to reduce the number of possible substances which can act at once, or by "fractionating" the test object, so as to study the effects of the serum on relatively simple organs, or, if possible, on simple tissues.

Let us further illustrate the unsuitability of short-cut methods for the solution of such problems. The question of the influence of a deranged selective power of the renal apparatus or of the gastro-intestinal mucosa in the genesis and maintenance of abnormally high blood pressure has often been discussed. It is conceivable that pressor substances may be absorbed in too great amount or excreted in too small an amount, or that the absorption of depressor substances may be defective, or their excretion excessive. A straightforward biological test calculated to appeal to the practical instincts of the clinician might seem to be afforded by the result of intravenous injection into dogs of specimens of urine from the same persons (figures 6 to 8). The injection of even considerable quantities is well borne. But the results will not lead us far in determining the presence or absence of particular pressor or depressor substances in the urine. A specimen collected from

T. on May 15th, 1909, on which occasion his serum gave a negative result, was without effect. But another specimen collected on April 26th, when his serum caused a fall of blood pressure, was also without effect. A specimen obtained on June 15th (figure 8), however, caused a distinct fall of blood pressure, which soon recovered to normal. A specimen obtained from C. on June 5th, when his serum caused a fall of blood pressure, produced a much greater and more lasting depression (figure 6). From another case of nephritis (S.), urine was obtained on May 27, 1909, which caused only a relatively slight fall of blood pressure (from seventy to fifty-five millimeters) when injected into the circulation of a dog, while blood plasma obtained at the same time produced a more distinct fall (from eighty to fifty-five millimeters). In about a minute, the blood pressure was again at its initial level. Here, then, in three specimens out of five the effect produced is a fall of blood pressure. The remaining two specimens caused no effect at all. Shall we then straightway conclude that all the statements which have been made as to the existence of pressor substances in urine, both normal and pathological, are erroneous? By no means. The body which Abelous[9] called "urohypertensine," and which Barger and Walpole[10] believe to be isoamylamine, is only one of several pressor bases produced in the putrefaction of proteins *in vitro*,[11] some of which are present in urine.[12] In all probability, they find their way into the urine after absorption from the intestine, where they are generated in the putrefaction of proteins. Abelous and Bardier[13] have indeed extracted "urohypertensine" from feces. Pressor substances then are present in urine. Whether they are diminished in amount in such conditions as are associated with high blood pressure, as is suggested by some observers (Abelous, in a case of arteriosclerosis; Bain, in gout), need not be discussed. The point I am making, and it is a very elementary one, is this: not only does a biological test, if it be on an entire organism, give no hint of the existence of these sub-

[9] Abelous and Bardier, *Jour. de physiol. et de path. gén.*, 1908, x, 627.

[10] *Jour. Physiol.*, 1909, xxxviii, 343.

[11] Abelous, Ribaut, Soulié and Toujan, *Compt. rend. Soc. de biol.*, 1906, lviii, 530; Rosenheim, *Jour. Physiol.*, 1909, xxxviii, 336; Barger and Walpole, *loc. cit.*

[12] Abelous, *loc. cit.*; Bain, *Lancet*, 1909, ii, 365.

[13] *Jour. de physiol. et de path gén.*, 1909, xi, 34.

stances, but when a positive result is obtained, it is in the opposite sense. The reason, of course, is that direct injection of a liquid like urine into the circulation is too crude a method for the detection even of pressor substances known to be present in it. It is necessary to separate the substances, to fractionate the urine, in order to demonstrate their effect, which would otherwise be masked by the dilution and by the opposing effects of other substances. What then of blood or serum, liquids at least as complicated as urine and in which adrenalin is not known certainly to exist?

The application of urine to the enucleated frog's eyeball in the hope of determining its adrenalin content, as was done by Pal,[14] who stated that human urine contained mydriatic substances, especially in nephritis, is not quite so unpromising a procedure as the injection of urine into the circulation, since the urine acts without dilution upon a relatively simple mechanism. Yet it is much too crude a method to give good results. For the urine itself is too complicated a liquid as well as too far removed from isotonicity, as Comessatti[15] justly remarks. He found that sodium chloride solutions can cause mydriasis in the frog bulb, though not so promptly as adrenalin, and concludes that a positive result obtained by this method with serum can at most raise the suspicion that adrenalin may be present. Waterman[16] also believes that the mydriatic action observed by Schur and Wiesel,[17] when urine and serum from pathological cases were applied to the frog eyeball, by no means indicates that adrenalin was present. W. H. Schultz[18] is also skeptical about the use of this method for the detection of adrenalin in the serum, and asks very pertinently: "If the excised eye yields uncertain results when immersed in a 1:625,000 Ringer adrenalin solution of known composition, what must one expect with a pathological serum the adrenalin content of which is undoubtedly less than this, not to mention the possible influence of other less well known substances,

[14] *Deutsch. med. Wchnschr.*, 1907, xxxiii, 1735.
[15] *Arch. f. exper. Path. u. Pharmakol.*, 1909, lx, 233.
[16] *Deutsch. med. Wchnschr.*, 1908, xxxiv, 1102.
[17] *Deutsch. med. Wchnschr.*, 1907, xxxiii, 2136.
[18] *Jour. Pharmacol. and Exper. Therap.*, 1911, i, 291. *U. S. Public Health and Marine Hospital Service*, 1909, Bul. 55.

that by their combined action may cause the slight mydriasis often observed with such sera?"

Direct injection of extracts of feces in salt solution in the hope of obtaining data on the mechanism of arterial hypertonus is even more straightforward and simple. The only difficulty is that it does not bring us the desired information. What conclusion, for example, can be drawn from the curve shown in figure 4 as to the existence in the feces of T. of substances connected with the maintenance of his persistently high arterial pressure? At 1 on the tracing, 2.2 cubic centimeters of a saline extract of feces obtained on May 15, 1909, were injected. The mean pressure fell slightly, to rise again to the original level or even a little above it. The fall of pressure was accompanied by marked acceleration of the heart. In figure 4, ten cubic centimeters of the same extract were injected into another dog. The course of the blood pressure curve was much the same as in figure 5, except that the fall of pressure was greater and more transient. At first view, it might be concluded perhaps by one who was anxious for positive results that the pressor substances in the larger amount of the extract quickly overcame the depressor substances, and brought the curve much more rapidly back to the normal level than when the smaller amount of extract was used. This explanation, however, would be quite erronous. What actually happened was that the large dose (it must be remembered, also, that it acted on another dog) paralyzed the respiration, and the rapid rise of blood pressure which occurs is the classical rise of asphyxia. It will be seen that the respiratory waves have disappeared from the curve. Slight spasms of the limbs were observed. The corneas were totally insensitive. Artificial respiration was tried, but the animal did not recover. In other experiments with fecal extracts from the same patient and from C., a fall of blood pressure was caused, followed by a rise to normal, but toxic symptoms did not supervene and the animal survived repeated injections. In figure 11 is shown the result of injecting two cubic centimeters of fecal extract from a normal man. It was observed that the heart was markedly accelerated and that the blood pressure fell sharply and then came back to normal or nearly so. A subsequent injection of the same amount of a similar extract from

another healthy man was followed by the death of the animal. The great variability in toxicity in all probability depends at least in an important degree upon the diet. It would be at once admitted by everyone that direct experiments of this kind could not inform us as to the content of the feces in pressor substances, although, as already mentioned, such are known to be present. Yet a filtered saline fecal extract is no more complex a liquid than serum, and, in the case of certain alien sera, no more toxic. Nor can any weight be given to general resemblances between two curves when we are attempting to decide whether the effects have been produced by one and the same substance or by different substances. For example, figures 8, 9, and 10 are fairly similar curves, and so are figures 7 and 11. The resemblance is quite as great as it often is between curves supposed to demonstrate the action of adrenalin in blood, and curves showing the effect of actual adrenalin solutions. Yet figure 8 represents the action of the urine of T., figure 9 that of the serum of C., and figure 10 that of the fecal extract of C. Hardly any one will conclude,I think, that the effect in the three cases is due to the same substance. Again, figure 7 illustrates the action on the blood pressure curve of C.'s urine, and figure 11 that of an extract of feces from a normal man. It would show even a greater degree of credulity in the matter of biological tests to imagine that the mechanism of the fall of pressure in the two cases was identical.

The results we have been discussing, although only of secondary interest in connection with the main subject of this paper, may yet possess some value in other relations, and are condensed in table I.

As far as is permitted by the limitations of our material, the following conclusions may be drawn:

1. Human serum injected intravenously into dogs has never produced any serious effect on the blood pressure. When an effect was obtained it was always a fall. Serum from the same person obtained at different times may produce different effects.

2. Human urine may cause a marked fall of pressure when injected intravenously into dogs, or it may have no effect. A rise of pressure has not been observed. Urine from the same person obtained on different days may produce different effects.

TABLE I.

Patient.	Date.	Effect of serum on blood pressure.	Effect of urine on blood pressure.	Effect of feces on blood pressure.	Effect of cerebro-spinal fluid on blood pressure.
T.	May 15, '09	0	0	Fall	——
T.	April 26, '09	Fall	0	——	——
T.	June 15, '09	——	Fall	——	——
C.	June 5, '09	Fall	Great fall	Fall	——
C.	June 22, '09	——	——	——	0
S.	May 27, '09	Fall	Slight fall	Fall	——
Normal man.	May 27, '09	——	——	Fall	——
Normal man.	May 27, '09	——	——	Marked fall	——
Fatal case of cyanide poisoning.		0	——	——	——

3. Saline extracts of human feces always cause a fall of pressure when injected into the circulation of dogs, sometimes with marked acceleration of the heart and stoppage of respiration. The toxicity of different specimens varies greatly.

4. There is no obvious relation between the effects on the blood pressure of dogs, of serum and urine obtained on the same date from the same person.

CONDENSED PROTOCOLS.

April 26, 1909. Specimens of blood and urine were obtained from T. and kept in an ice chest until clear serum had separated from the clot; then the effect on arterial pressure of intravenous injection into a dog weighing 9 kilos was tested (figures 1 and 2). As in all the observations, the animal was anesthetized with morphine and ether. The serum produced a small and transient fall of blood pressure following the slight preliminary rise due to the mechanical effect. Three successive injections of filtered urine, each of 10 c.c., produced the same slight rise as a similar amount of sodium chloride solution.

May 15, 1909. Blood, urine, and 200 gms. of feces were obtained from T. The feces were extracted with 0.85 per cent. sodium chloride solution, and the extract was filtered. The material was kept in an ice chest until clear serum had separated from the blood clot. Then the effect on blood pressure was determined on a dog weighing 8 kilos (figures 3 to 5). The slight and momentary rise of pressure caused by injection of 10 c.c. of the serum is exactly similar to that produced by sodium chloride solution, and is not succeeded by a fall. A subsequent injection of 8 c.c. of serum (about 1¼ minutes later) gave exactly the same result. At 4 (figure 4), 10 c.c. of the fecal extract was injected. The pressure fell and then rose again to the normal height. It was noticed that the animal had stopped breathing. Slight spasms of the limbs were observed. Artificial respiration failed to restore the animal. Blood taken from the heart at autopsy was imperfectly clotted after standing over night in the ice chest, but on pouring some of the plasma on a strainer it quickly clotted. Evidently the plasma contained plenty of

fibrinogen, which perhaps was restrained from clotting by an anticoagulant action of some constituent of the fecal extract. Very little hemolysis had occurred, as only a trace of blood pigment was present in the plasma (in the form of oxyhemoglobin). On the next day, fecal extract and urine were intravenously injected into a 7 kilo dog. At 1 (figure 5) 2.2 c.c. of the extract were injected. The pressure fell, but soon recovered to the original level or a little above it. A subsequent injection of 10 c.c. of urine caused a slight and transient rise of pressure, the same rise as was caused by an equal amount of sodium chloride solution. Otherwise there was no noticeable effect. A second injection of 2.2 c.c. of fecal extract was followed by a moderate temporary fall of pressure, and then by marked dyspnea with slight spasms of the limbs, as in yesterday's experiment, and finally by death. Blood from the animal kept over night in the ice chest clotted only partially and slowly.

June 15, 1909. Urine was collected from T. and injected into the femoral vein of a 5 kilo dog; a distinct but transient fall of pressure occurred (figure 8). The urine still contained a considerable amount of albumin. It was very pale, and its specific gravity was 1006.5.

Urine obtained the same day from another man (C.) with still higher blood pressure but no albumin in the urine caused a greater and more lasting depression (figures 6 and 7), although eventually the pressure returned to normal. It was noticed that the heart was accelerated after the injection. The specific gravity of C.'s urine was 1016. It was much more highly colored than T.'s. Repeated injections of urine from these two cases caused no permanently harmful effects. Injections of extracts of feces from the two cases made with 0.85 per cent chloride solution, on the same day as the urine was collected, caused a fall of pressure not much greater but more persistent than that caused by the urine. In all, 14 c.c. of fecal extract and 30 c.c. of urine were injected in the course of an hour. Yet the animal had to be killed at the end of the experiment. The fecal extract was made by extracting 100 gms. of feces from each patient with 125 c.c. of salt solution. At this time, after the administration of sodium silicate, the patients had a lower blood pressure than for some time. As it was scarcely conceivable that sodium silicate could affect the blood pressure in any other way than by its alkalinity, the idea was suggested that an acidosis, associated with deficient transporting power of the blood for carbon dioxide, and, therefore, with increased stimulation of the vasomotor mechanism by carbon dioxide, might be a factor in maintaining the high blood pressure. T. was accordingly put on large doses of sodium bicarbonate, all other medication being stopped. This treatment was associated with a fall of blood pressure to a level distinctly lower than at any time since his admission. He had been treated with nitroglycerin with little effect on the blood pressure. The bicarbonate treatment was continued for a week, during which he said he felt better than for some time. The bicarbonate was then stopped. The blood pressure increased and he felt worse. A further course of bicarbonate produced the same favorable subjective effects and the same fall of blood pressure. Eventually, however, it had to be stopped, as his digestion was impaired. Of course no great stress is to be laid on such an experiment, yet the change in the blood pressure records was remarkable. It seems possible, therefore, that in some cases a factor in increased blood pressure may be diminished alkalinity of the circulating liquids.

June 5, 1909. Five c.c. of blood serum from C. caused a decided though transient fall of pressure when injected intravenously into a small dog (figure 9). The respirations became deep and slow. After section of both vagi, no fall of pressure was caused by a further injection of the serum, nor any rise, except a small one due to the mechanical effect. Injection of urine produced a great fall of pressure, which returned only very slowly toward the normal, just as in the experiment of June 16 (figure 7). With fecal extract the fall of blood pressure was decided but more transient than with urine (figure 10), and the fall occurred after as well as before division of both vagi. After receiving 13 c.c. of serum, 8 c.c. of urine, and 9 c.c. of fecal extract (100 gms. of feces extracted with 125 c.c. of salt solution), the animal was in good condition and had to be killed. Blood obtained from the carotid clotted normally in a few minutes. The separated serum was tinged with hemoglobin distinctly though not deeply.[10]

May 28, 1909. Blood was obtained on the preceding day from S., a nephritic patient. The blood did not clot, and plasma in large amounts separated from it in the ice chest. 1.30 P. M. Injections were begun into the femoral vein of a male puppy, weighing 5 kilos. Injection of 5 c.c. of plasma caused a slight rise of pressure succeeded by a fall of 25 mm. In about one minute the pressure had returned to the initial level (80 mm.). A second injection of 5 c.c. of plasma caused a much smaller and more transient fall of pressure, while a third injection of 7 c.c. produced about the same effect as the first injection. Injection of 7 c.c. of urine from S. produced no noticeable effect on the curve. Two subsequent injections of 10 c.c. each caused a slight and transient fall (of 15 mm., the initial pressure being 70 mm.).

Subsequent injection into the same dog of 7 c.c. of human plasma from a case of suicide by cyanide poisoning had practically no effect. Then 4 c.c. of fecal extract from S. were injected (made by extracting 40 gms. of semi-solid feces with 50 c.c. of 0.85 per cent. sodium chloride solution and filtering). The extract had only a slight odor. The pressure fell from 80 to 30 mm. A subsequent injection of 4 c.c. of the extract lowered the pressure from 62 to 45 mm.; a further injection of 9 c.c. lowered it from 60 to 35 mm. A final injection of 4 c.c. of the extract had practically no effect on the pressure, which at 3.30 P. M. had risen to 80 mm. The animal had, under anesthesia, to be killed by bleeding. The blood clotted in a few minutes.

May 27, 1909. Saline extract of feces was obtained from two normal men. Figure 11 shows the effect of injecting 2 c.c. of one extract into the vein of a dog weighing 5 kilos. The blood pressure eventually came back almost to the initial height. After subsequent injection of 2 c.c. of the other extract, the heart immediately became much accelerated, the blood pressure fell greatly, and the animal died. The extracts had only a slight odor.

[10] This agrees fairly well with an incidental observation of Külbs, *Arch. f. exper. Path. u. Pharmakol.*, 1906, lv, 87, that filtered extracts of human feces made with an equal volume of salt solution cause no hemolytic effect when injected intravenously into rabbits, even when *in vitro* their action is strongly hemolytic. On the other hand, either his statement that rabbits bear very well a single injection of 2 to 6 c.c. is based on a very limited number of observations, or rabbits are much more resistant than dogs. As mentioned before, for dogs some specimens of feces are apparently almost harmless, others are very toxic.

To come back to the point from which we started in discussing the biological tests for adrenalin, it is clear that the best results will be obtained: (1) by using not entire animals, but such isolated organs or parts as are specifically acted on by adrenalin, or in other words, if the expression is permissible, by fractionating the test organism; (2) by fractionating the liquids to be tested as far as possible, so as to exclude other possibly active substances than adrenalin, for example, removing the proteins from serum, as Schlayer did. He showed[20] that removal of protein from a serum did not weaken its action on strips of vessels. Very slight reflection shows that in general with such quantities of material as are at our disposal for clinical investigations, and in consideration of the nature of the material, analysis is far more feasible in the first than in the second direction. It behooves us, therefore, to multiply as far as may be the number of different test objects to which a liquid suspected to contain adrenalin may be applied. There is a pretty general agreement that chemical tests are hopeless for the quantities of adrenalin which can possibly be present in the general blood stream. Comessatti[21] indeed gives a color reaction (rose color with mercuric chloride) for adrenalin, which he recommends for testing urine, as it excludes brenzcatechin, a substance which may be confounded with it if the well known ferric chloride reaction is employed, as was the case, for example, in the work of Schur and Wiesel.[22] But, according to Waterman,[23] Comessatti's sublimate test is not in all respects satisfactory when applied to serum. Neither Fraenkel nor anybody else, as far as I am aware, has employed two methods in the same series of experiments and on the same specimens of blood or serum. Even if it were true that in one laboratory a given specimen of human serum gave a reaction with a strip of ox artery resembling that given by adrenalin, and that in another laboratory a specimen of another human serum gave a reaction with rabbit uterus similar to that given by adrenalin, the evidence for the presence of adrenalin in both specimens of

[20] *München. med. Wchnschr.*, 1907, liv, 1898; 1908, lv, 2604; *Deutsch. med. Wchnschr.*, 1907, xxxiii, 1897.

[21] *München. med. Wchnschr.*, 1908, lv, 1926.

[22] *Deutsch. med. Wchnschr.*, 1907, xxxiii, 2136.

[23] *Arch. f. d. ges. Physiol.*, 1909, cxxviii, 48.

serum would be much less convincing than if the two reactions had been obtained under similar conditions for each serum. Recently a paper by W. B. Cannon and D. de la Paz[24] has appeared on the detection of adrenalin in the blood of cats previously subjected to strong emotional disturbance, by means of its effect upon the longitudinal muscles of the small intestine studied by the method of Magnus.[25] In these experiments no error was introduced by the application of alien blood or serum to the test object, since it was the cat's own small intestine that was employed. Also the comparison of blood from animals after removal of the adrenals with blood from normal animals subjected to the same emotional stimuli, in a manner "fractionated" out the adrenal contribution and therefore rendered it more feasible to rely on a single biological test object.

It seems to me that the combination of this reaction (even with the disadvantageous condition inseparable from observations on human blood of having to employ a heterologous test object) with a reaction in which the effect of sympathetic stimulation is to cause contraction of smooth muscle, affords a more reliable clinical test for adrenalin than any single test object can afford. It is, of course, as has already been indicated, theoretically desirable that a liquid which is being tested for adrenalin should be subjected to all the reactions which adrenalin is known to give. In practice, however, the permissible number of tests is very much limited, apart altogether from the amount of time required, by the amount of material which can be obtained. Having already worked with the uterus test, I chose this to combine with the intestine reaction, obtaining both objects from the same rabbit. It is unquestionably a drawback, as Cannon remarks, that only half the animals are available for the uterus tests. The field, indeed, is even more restricted, since adult animals are required, the spontaneous contraction of uterus rings from sexually immature female rabbits being too feeble and uncertain. But once an animal suitable for the uterus test is in hand, the institution of the intestine reaction scarcely adds at all to the time consumed, since the intestine is ready almost at once and the

[24] *Am. Jour. Physiol.*, 1911, xxviii, 64.
[25] *Arch. f. d. ges. Physiol.*, 1905, cviii, 48.

test can be carried out on it while the uterine contractions are developing to the most favorable point. It is probable that the perfusion test, as applied quite recently by P. Trendelenburg[26] to the frog circulation (which is also a test in which smooth muscle is caused to contract by adrenalin), would be a still better one than the uterus test to combine with observations on the intestine. Injection of blood or serum into the circulation of mammals, as is well known, is not suitable for determining the presence or absence of adrenalin, since the amount of available material must be far too greatly diluted, and effects are produced on the heart and on other mechanisms which tend to mask any true adrenalin action which might be present (see protocol of June 5, 1909, p. 388).

Method.—The method employed was that in common use. The rabbit was killed by being struck on the back of the neck. The small intestine and uterus were immediately removed and the contents of the intestine washed out with Ringer's solution (Locke's modification). Usually the intestine was taken out in several pieces after double ligation wherever it was to be cut. The tissues were at once transferred to a vessel containing Ringer's solution immersed in a large bath of water kept at a constant temperature (39° C.) by means of a calcium chloride regulator, the water being constantly stirred by a mechanically actuated stirring arrangement. Oxygen bubbled through the Ringer's solution. Portions of intestine about 2 or 2½ cm. in length, and portions of uterus, usually of one of the horns, about 1½ to 2 cm. in length were attached to one arm of a double lever writing on a slowly moving drum, so that the longitudinal fibers exerted a pull on the lever. A small platinum ring was fastened to one end of the preparation by a thread passed through the outer coat by means of a needle. This ring was engaged by a small hook attached to a rubber cork which closed the bottom of the small glass cylinder in which the preparation was immersed. The capacity of the cylinder was 7 c.c. The upper edge of the preparation was caught by a *serre fine*, a thread attached to which was connected to the lever by a hook. The small cylinder was supported in the bath. Near it was suspended a thermometer.

I do not desire at present to go into the question of the adrenalin content of pathological serum, further than to quote a protocol illustrative of the results obtained with serum from T. (the case of nephritis with persistently high blood pressure, and albumin and casts in the urine, already mentioned).

September 13, 1909. Blood was obtained from the arm of T.
The blood was obtained by puncture of a vein and allowed to clot. It was

[26] *Arch. f. exper. Path. u. Pharmakol.*, 1910, lxiii, 161.

kept in the ice chest till clear serum had separated. Observations were then made on rabbit uterus preparations. Some of the curves are reproduced in figures 12 to 14.[27] The rabbit was full grown but not pregnant. Portions of a horn were used. Spontaneous contractions commenced a few minutes after the preparation was attached to the lever. Further description of the experiment is given below (figures 12 to 14).

The general resemblance between the action of the serum and that of adrenalin should carry, I believe, but little weight, in the absence of tests with at least one other test object.

My observations on normal sera show at once how the application even of two properly chosen biological tests tends severely to retrench the number of positive results. Take for example figure 15, a tracing from a rabbit uterus preparation in dog serum. At 1, the Ringer's solution was removed. At 2, the dog serum was added, and at once there was a great increase of tone. This quickly reached a maximum and then declined below the original level. The contractions disappeared, but were later resumed while the preparation still continued in the serum (not shown in the figure). The uterus was from a rabbit not very far advanced in pregnancy.

If one had been content with the uterus test alone, a positive conclusion might have been drawn as to the presence of adrenalin in the dog serum. The application of the intestine reaction (figure 16), however, shows that this conclusion would not be justified. At 3, the Ringer's solution in which the rabbit intestinal segment was beating was replaced by the dog serum. Had the increase of tone in the uterus in figure 15 been due to adrenalin, signs of inhibition might have been expected in the intestinal preparation, *i. e.*, diminution of tone with temporary stoppage or slowing of the contractions. Instead of this, the tone rapidly augmented, the contractions continuing at a rate at least as great as in the Ringer's solution. By and by the tone began to diminish again. The contractions maintained their rate but increased markedly in strength while the preparation continued in the serum, as shown on the lower curve which cuts the curve traced while the tone was increasing. On replacing the serum again by Ringer's solution (figure 17), the tone diminishes somewhat, while the contractions proceed

[27] Portions of these figures were published in my Manual of Physiology, 6th edition, 1910, figure 449, p. 1026.

without sensible change of rate. In figure 18, it is seen that the contractions are going on with great regularity and at a somewhat accelerated rate in Ringer's solution.

Even with the two tests, then, we can conclude that no evidence has been obtained of the existence of a detectable amount of adrenalin in this dog serum. I do not intend to go into the question as to what the active substance or substances are which produce the increased tone in both uterus and intestine, nor into the question whether such biological tests are of more value for some kinds of alien serum than for others. Crude they must be to some extent, for no alien serum can be supposed to be indifferent to the uterus or intestine of a rabbit. Had the slate been clean, it certainly would not have appeared to me a promising attempt to write on it a method for the detection, let alone the assay of adrenalin, depending upon the exposure of animal tissues to liquids at once so complex and so highly specific as foreign sera. The assay of adrenalin, when adrenalin is known to be present in a simple solution, is an entirely different matter. The object of the present paper is strictly limited to pointing out how without greatly increased labor some of the so-called biological tests can be rendered more nearly crucial.

In figure 19, with an intestinal preparation from another rabbit and serum from another dog, the increase of tone produced by the serum is even more striking than in figure 16. It is also more persistent. There is no trace of preliminary inhibition. The contractions continue during the period of increased tone, and indeed their frequency is somewhat augmented. A fresh preparation of intestine from the same rabbit (figure 20) gave a similar great increase of tone, the lever rising above the top of the drum. With the uterus preparation (figure 21), a slight increase of tone was caused by this dog serum, but much less than that caused by the serum of the other dog in figure 15. The rate of contraction was also somewhat accelerated for two or three beats. The increase of tone soon culminated, and then disappeared, the line of minima falling indeed somewhat below the original level. The rabbit was a virgin and probably not quite mature. Here again the two biological tests instituted would certainly not permit the conclusion that adrenalin was present in this dog serum.

Incidentally this experiment confirms the statement of Kurdinowski[28] that the virgin uterus when subjected to an artificial circulation with Ringer's solution can execute spontaneous contractions, and the similar statement of Cushny[29] for the virgin uterus *in situ*. For it shows that even excised portions of a uterus not yet sexually mature can contract spontaneously.

[28] *Arch. f. Physiol.*, 1904, Supplement, 323.
[29] *Jour. Physiol., loc. cit.*

In figure 22 is reproduced a portion of the curve obtained when the Ringer's solution in which a rabbit intestinal preparation had been contracting was replaced by blood from a normal man thirty-four years of age. The blood was obtained by inserting a large hypodermic needle, previously sterilized in oil, into a vein on the back of the hand, and was at once defibrinated. Since the quantity of blood procured (14 c.c.) was not sufficient to permit the serum to be used for as many experiments as were planned, the entire defibrinated blood was employed.

In figure 22, it is seen that normal human blood produces the same marked increase in tone in the rabbit intestine preparation as is produced by dog serum. The contractions are not inhibited at all, but go on well throughout the whole period of immersion. It proved impossible to get good spontaneous contractions from the uterus preparation from this rabbit, which was well advanced in pregnancy. Several segments of the uterine horns, especially the portions between two ova and also the body of the uterus were tried, but in vain. Next day, however, the blood having been kept over night in the ice chest, good uterine preparations were obtained from another rabbit five weeks after parturition, and on these the influence of the normal human blood and that of dilute adrenalin solutions were compared.

Figure 23 shows the effect of human blood, and figure 24 that of adrenalin (1: 500,000 in Ringer's solution). The curves reproduced in the two figures were taken from the same preparation, figure 23 being first obtained and the blood then thoroughly washed off with Ringer's solution before the adrenalin solution was added. In both cases it would seem that the first effect was an increase of tone with stoppage of spontaneous contractions. Very soon the tone began to diminish again and the spontaneous contractions returned (not shown in figure 23), although the return was later in the blood than in the adrenalin solution. In both cases the spontaneous contractions were feebler in the blood than in the Ringer's solution. In figure 24, at 11 a stronger solution of adrenalin (1: 100,000 in Ringer's solution) caused a decided diminution of tone with perhaps a very slight preliminary increase. On the whole, to the eye the resemblance between the effects of blood and adrenalin solution in the two figures is striking and on a superficial view it might suggest that the cause was the same, *i. e.*, that the blood contained adrenalin. The application of the second test (with the intestinal preparation) at once shows that this conclusion is premature. Figure 25 illustrates the effect of replacing the Ringer's solution with the blood; *viz.*, the same decided increase of tone which was obtained with perfectly fresh blood. Incidentally this excludes the possibility that in the twenty-four hours elapsing since the human blood was drawn, the adrenalin in it might have disappeared. For as regards the intestine, its behavior was the same two hours after withdrawal from the body.

Figure 26 shows the effect of adrenalin (1: 1,000,000 in Ringer's solution) on the same preparation of intestine after the blood had been thoroughly washed off with Ringer's solution. There is a diminution of tone which gradually reaches a minimum and then slowly increases toward the original level without, however, in the time of observation quite reaching it. In figure 27, with a stronger solution of adrenalin (1: 100,000) the same preparation underwent a very marked diminution in tone. In both cases the spontaneous contractions persisted, although diminished somewhat in force. The effect then of the normal

human blood, although quite similar to that of adrenalin when the uterus preparation is alone regarded, is entirely different from that of adrenalin when the test is made on the intestine. Again, it is obviously proper to conclude that the combined result of these two tests is negative as regards the presence of adrenalin in normal blood.

In some pathological cases, certain observations were made on other possible factors in maintaining high blood pressure besides the presence of specific pressor substances in abnormally great amount.

June 22, 1909. T. Patient lying in bed. The systolic blood pressure was estimated by the Riva-Rocci sphygmomanometer, as shown in the following table.

TABLE II.

Blood pressure during natural breathing.	Blood pressure during forced breathing.	Number of respirations in period of forced breathing.	Blood pressure after the period of forced breathing.
210 mm. Hg.	210	15	215
210	200	20	195*
205	190	20	200*
200	195	15	200*
200	185	15	190†
200	190	190	190†
			210‡

* One minute after forced breathing stopped.
† Two minutes after forced breathing stopped.
‡ Three minutes after forced breathing stopped.

The forced breathing was not followed by complete apnea, but by hypopnea. The period of forced breathing could not be made longer because unpleasant symptoms (dizziness) appeared.

September 11, 1909. T. lying in bed.

	Blood pressure.
Breathing naturally	205, 205, 205 mm. Hg.
Breathing through mask (at end of 10 deep respirations)	200
Breathing through mask (at end of 20 deep respirations)	195
Breathing through mask (at end of 30 deep respirations)	190
Breathing oxygen through mask (respirations of the same depth as before with the mask on)	190
Mask taken off; breathing air; normal depth of respiration	195
Mask on; breathing oxygen	190, 195
Mask on; breathing air; normal depth of respiration	200
Mask on; breathing a mixture of oxygen and carbon dioxide	220
Mask on; breathing air	185, 200, 185
Mask on; breathing a mixture of oxygen and carbon dioxide (proportion of carbon dioxide less than before)	190, 180

Mask taken off; breathing air 190
Mask on; breathing a mixture of oxygen and carbon dioxide (probably about as much carbon dioxide as in the first carbon dioxide experiment). 185, 185, 190

The object of these experiments was, in conjunction with adrenalin estimations in the blood, to analyze the conditions on which the high blood pressure depends, by determining whether the washing out of carbon dioxide or the increase of the alveolar oxygen partial pressure has any effect on the blood pressure in this condition. It will be seen from the first protocol that forced breathing causes practically always a slight but definite fall of blood pressure. The second protocol indicates that the washing out of carbon dioxide is a factor in the fall of pressure, since the highest pressure was obtained during respiration of a mixture rich in carbon dioxide.[30] This does not, however, exclude the mechanical influence of forced respiration, which according to Hill and Flack[31] causes a fall of blood pressure in normal persons and which I have shown to be a factor in the diminution in the rate of blood flow (in the hand) associated with forced respiration.[32] No clear indication of any action of oxygen on the blood pressure is afforded by the protocols, which agrees with my negative result on the blood flow through the hand in normal persons.[33]

Since increased intracranial pressure causes increased systemic arterial pressure, it seemed desirable to test the question whether the withdrawal of cerebrospinal fluid would affect the blood pressure.

Observations were accordingly made on C. with the Riva-Rocci sphygmomanometer on June 22nd, 1909, before, during, and after the withdrawal of cerebrospinal fluid by lumbar puncture. The puncture was performed by Dr. Richard Dexter, to whom I am much indebted for constant assistance at the hospital during this investigation. The syringe was connected with a manometer tube in which on the passage of the needle into the spinal canal the cerebrospinal fluid rose to a height indicating a pressure of 150 mm. of the fluid.

[30] This is, of course, not at variance with the recent statement of Hooker (*Am. Jour. Physiol.*, 1911, xxviii, 361) that the direct action of carbon dioxide on the perfused blood-vessels of the frog is a diminution of vascular tone. In the intact body, the action on the central mechanisms may mask any peripheral effect.

[31] *Jour. Physiol.*, 1910, xl, 347.

[32] Stewart, *Am. Jour. Physiol.*, 1911, xxviii, 190.

[33] Stewart, *Jour. Pharmacol. and Exper. Therap.*, 1911, ii, 477.

TABLE III.

Time.	Blood pressure.	Notes.	Time.	Blood pressure.	Notes.
3.20 P.M.	230	These two readings made by independent observers.	3.40 P.M.	185	
3.23 "	230		3.45 "	195	
3.25 "	215	Needle inserted into spinal canal.	4.00 "	175	
3.25 "	190	After beginning to draw off liquid.	4.07 "	180	Pulse 100.
	190		4.15 "	180	
	190	Fluid began to run better from cannula.	4.30 "	175	
			4.45 "	175	
	200		5.00 "	176	
	190		6.00 "	176	
	195	Removed the needle.	7.00 "	165	
3.38 "	195		8.00 "	160	
	195		6.00 A.M.	160	

The patient complained that after the puncture he could not see so well with his left eye. Ophthalmoscopic examination did not reveal any retinal hemorrhage. Retinitis was present before and remained unaltered. The cerebrospinal fluid drawn off was perfectly clear. The quantity was 8 c.c. The blood pressures observed on this patient, after the puncture, were much the lowest that he had shown since admission. Subjectively, however, his condition was, if anything, worse. He complained of headache.

Intravenous injection of the cerebrospinal fluid into a dog weighing 3,750 gms. (anesthetized with morphine and ether) produced exactly the same slight effect on the arterial blood pressure as injection of the same amount of sodium chloride solution.

SUMMARY.

1. The combination of a biological test object on which adrenalin causes contraction of smooth muscle (perfused blood-vessels or the uterus in certain conditions) with a biological test object on which it produces inhibition of contraction of smooth muscle (intestine) greatly diminishes the chance of error in testing blood (or other body liquids) for adrenalin. A control experiment with adrenalin solutions should, in general, accompany each observation on the blood.

2. When properly chosen biological tests are employed, no evidence is obtained of the presence of adrenalin in detectable amount in normal blood taken from the general circulation.

3. In a case of nephritis with albuminuria and persistently high arterial pressure, the pressure was diminished by forced breathing. The washing out of carbon dioxide seemed to be a factor in this diminution as well as the mechanical interference with the circula-

tion. In this case, the administration of large doses of sodium bicarbonate was associated with a marked diminution in the blood pressure.

4. In another case with persistently high blood pressure, the drawing off of cerebrospinal fluid caused a distinct diminution in the arterial pressure, presumably by lowering the intracranial pressure. No pressor substance was detected in the cerebrospinal fluid.

EXPLANATION OF PLATES.

All figures are to be read from left to right. In figures 1 to 11 the time trace marks seconds. The base line, where not otherwise indicated, corresponds with the time trace. Figures 12 to 27 have been reduced one half.

PLATE 34.

FIG. 1. Effect on blood pressure of injection of 10 c.c. sodium chloride solution.

FIG. 2. Effect on blood pressure of 10 c.c. human serum (from T., a nephritic patient).

FIG. 3. At 1, injected 10 c.c. sodium chloride solution. At 2, 10 c.c. human serum (from T.).

FIG. 4. Effect of injection of fecal extract (from T.) on blood pressure.

FIG. 5. Effect of injection of fecal extract (from T.) on blood pressure.

PLATE 35.

FIG. 6. At 1, injected 5 c.c. urine from C, a patient with persistently high blood pressure.

FIG. 7. A 5, injected into the same dog as was used for figure 6, 9 c.c. urine from C.

FIG. 8. At 2, injected 7 c.c. urine from T. into the same dog as was used for figures 6 and 7.

FIG. 9. At 3, injected 5 c.c. serum from C.

FIG. 10. At 6, injected 1 c.c. saline extract of feces from C. into the same dog as was used for figure 9.

PLATE 36.

FIG. 11. At 2, injected 2 c.c. saline extract of feces from a normal man.

FIG. 12. Rabbit uterus preparation. The rabbit was fully grown and not pregnant. At 1, the Ringer's solution in which the preparation had been contracting was replaced by blood serum from a patient T., a man with persistently high arterial pressure who was suffering from renal disease. The tone was markedly increased and the contractions stopped at first, but soon recommenced (at 5), the tone falling somewhat, but remaining greater than in the Ringer's solution. The rate of contraction is somewhat greater in the serum than in the Ringer's solution. Time trace, half minutes.

FIG. 13. Upper trace, rabbit uterus preparation contracting in serum from

T. diluted with its own volume of Ringer's solution. The lower trace is from the same preparation contracting subsequently in Ringer's solution after the serum had been washed off. The contractions in the Ringer's solution are much feebler than in the serum, although more frequent. Time trace, half minutes.

Fig. 14. Uterus preparation from the same rabbit as was used for figures 12 and 13. At 1, a little adrenalin was added to the Ringer's solution. The preparation was contracting spontaneously (not shown in figure). The adrenalin caused at once a great increase in tone and the contractions stopped for a time. At 2, the drum was stopped till the spontaneous contractions began again, which they did after the tone had gradually decreased to the extent shown on the tracing. Time trace, half minutes.

Fig. 15. Rabbit uterus preparation contracting in Ringer's solution, which was removed at 1 and replaced at 2 by the serum of a normal dog. There was a great increase of tone and the rhythmical contractions ceased for a time. The increase of tone soon gave place to a diminution below the initial level. Time trace, five second intervals.

Fig. 16. Intestine preparation from the rabbit whose uterus was used for figure 15. At 3, the Ringer's solution in which the preparation was contracting was replaced by the dog serum. The tone was much increased, the contractions persisting. 4 shows the continuation of the tracing, the preparation remaining in the serum after the tone had diminished again. Time trace, five second intervals.

Fig. 17. Tracing continued with same intestine preparation as in figure 16. At 6, began to remove the dog serum. At 7, completed removal of the serum. At 8, added Ringer's solution. Time trace, five second intervals.

Fig. 18. Tracing continued with the same intestine preparation, as in figure 17, the intestine contracting in the Ringer's solution after removal of the dog serum. Time trace, five second intervals.

Fig. 19. Intestine preparation from another rabbit and serum from another normal dog. While the preparation was contracting spontaneously in Ringer's solution, the solution was removed at 1 and dog serum added at 2. Immediate increase of tone resulted, the contractions continuing. Time trace, half minutes.

Fig. 20. Another preparation from the intestine of the same rabbit as was used for figure 19. Preparation contracting in Ringer's solution. At 1, began to remove Ringer's solution. At 2, added dog serum. The increase of tone was so great that the writing point rose above the top of the drum. Time trace, half minutes.

Plate 37.

Fig. 21. Uterus preparation from the same rabbit as was used for figures 19 and 20. Contracting spontaneously in Ringer's solution. At 1, removed the Ringer's solution, and at 2, added dog serum. Time trace, half minutes.

Fig. 22. Intestine preparation from another rabbit, contracting in Ringer's solution. At 1, removed the Ringer's solution, and at 2, added fresh blood from a normal man. Marked increase of tone with decided quickening in the rate of contraction. Time trace, half minutes.

FIG. 23. Rabbit uterus preparation contracting in Ringer's solution. At 1, removed the solution, and at 2, substituted for it normal human blood. Transient slight increase of tone followed by gradual diminution below the initial level and cessation of contractions. Time trace, half minutes.

FIG. 24. Same uterus preparation as was used in figure 23. The spontaneous contractions were resumed while the preparation was still in the blood. At 6, removed blood, and at 7, added adrenalin (1: 500,000). Immediate transient increase of tone resulted, followed by diminution. Note resemblance between figures 23 and 24. At 10, the adrenalin solution was removed with the drum stopped. At 11, a stronger adrenalin solution (1: 100,000 in Ringer's solution) was substituted. Time trace, half minutes.

FIG. 25. Rabbit intestine preparation contracting in Ringer's solution. At 1, removed the solution, and at 2, added human blood (kept 24 hours in ice chest). Time trace, half minutes.

FIG. 26. Same preparation of rabbit intestine as was used in figure 25, after removing the blood and washing several times with Ringer's solution. Preparation beating in Ringer's solution. At 4, removed the solution, and at 5, substituted adrenalin (1: 1,000,000 in Ringer's solution). Diminution of tone. Time trace, half minutes.

FIG. 27. Another preparation of intestine from the same rabbit as was used in figure 26. Contracting in Ringer's solution. At 4, removed the solution, and at 5, substituted adrenalin solution (1: 100,000). Marked diminution of tone. Time trace, half minutes.

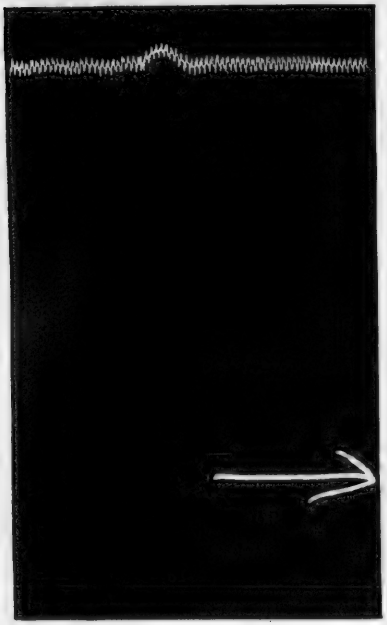


FIG. 1.

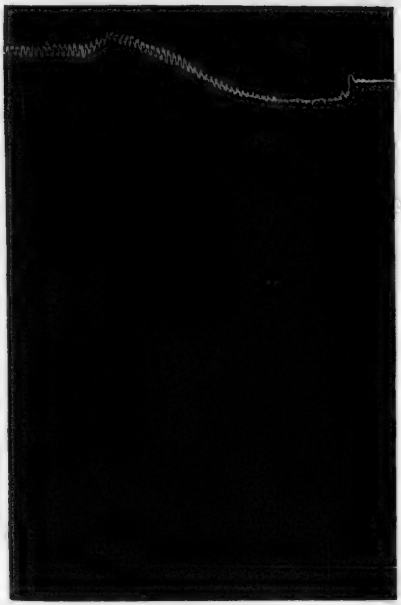


FIG. 2.

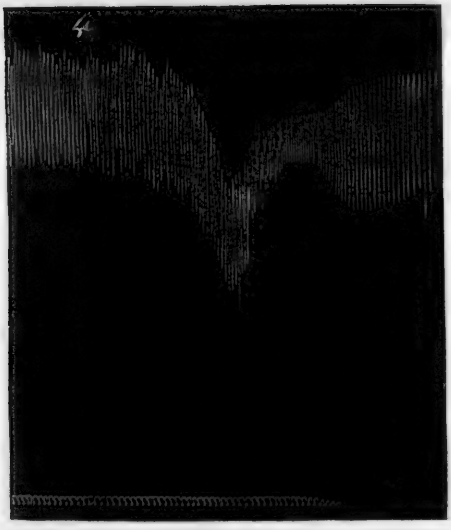
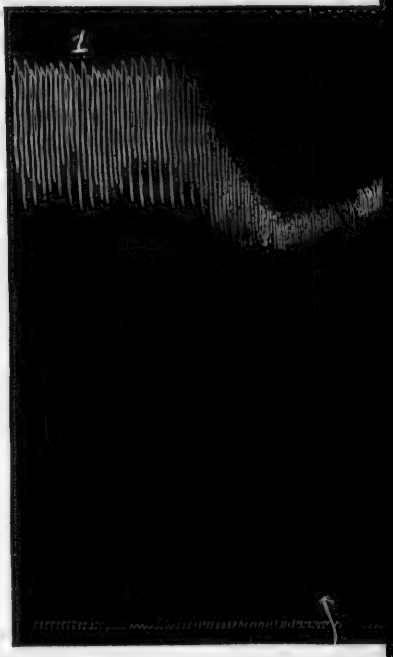


FIG. 4.



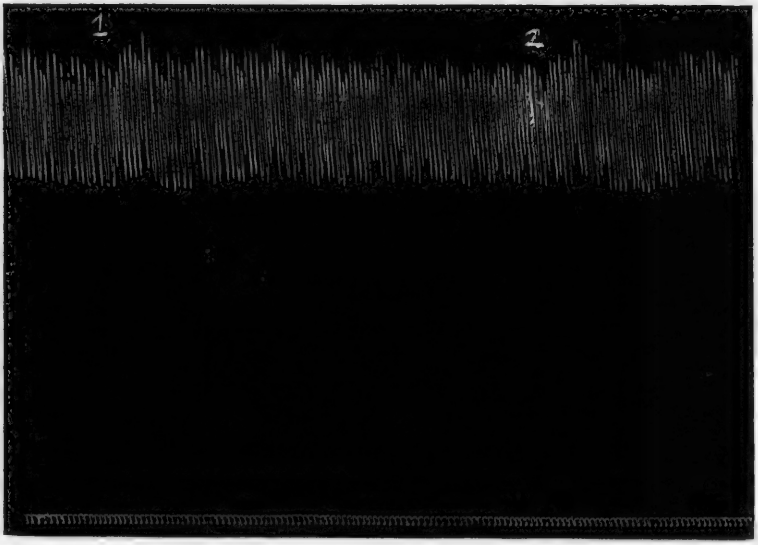


FIG. 3.

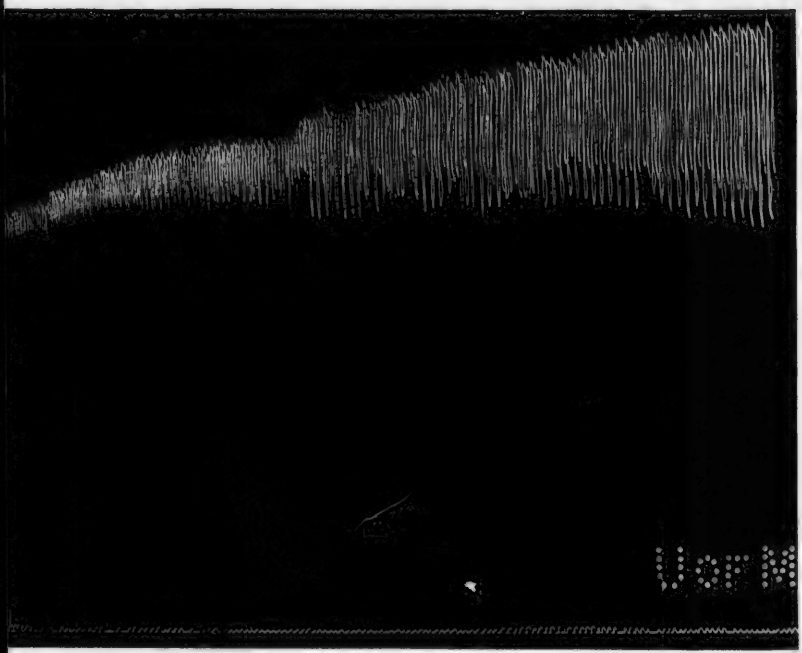


FIG. 5.

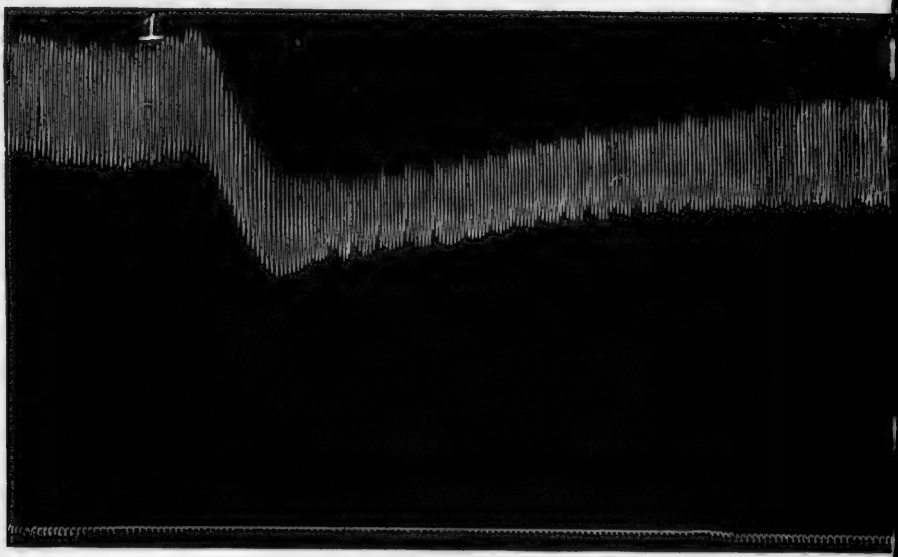


FIG. 6.

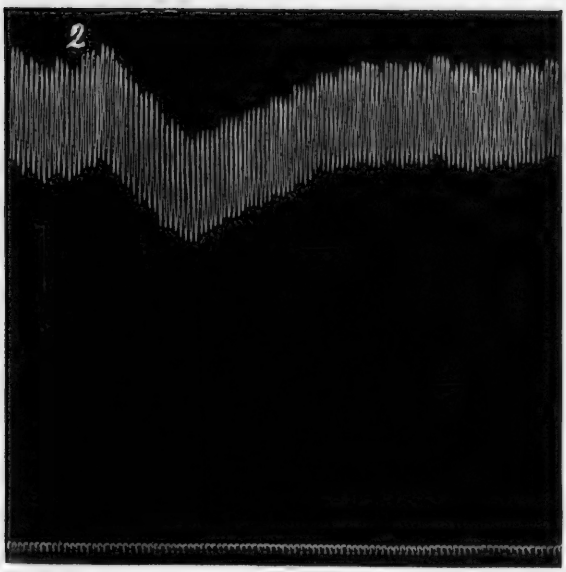


FIG. 8.

FIG. 9.

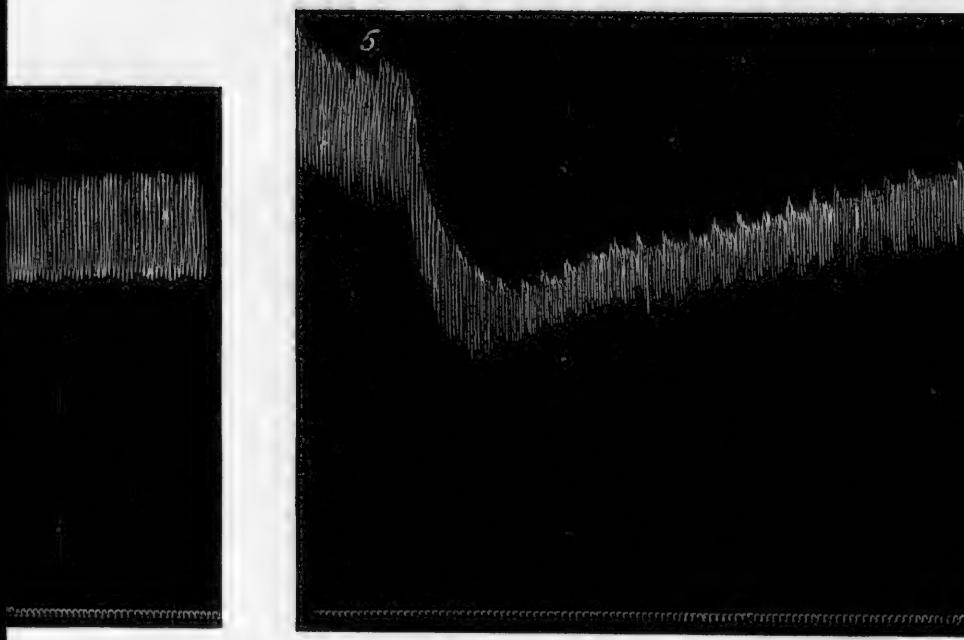


FIG. 7.

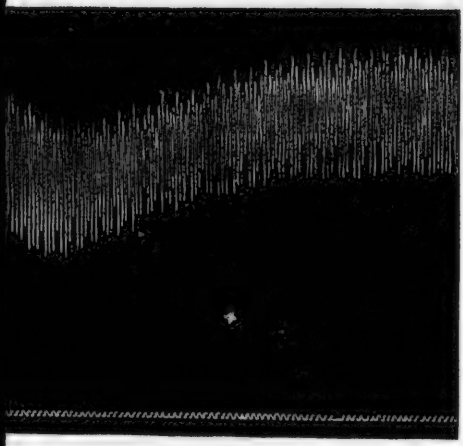


FIG. 9.

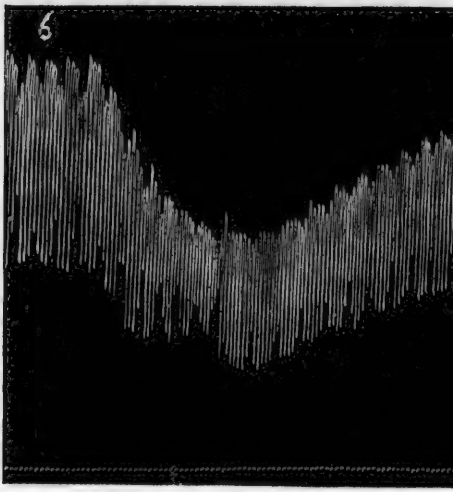
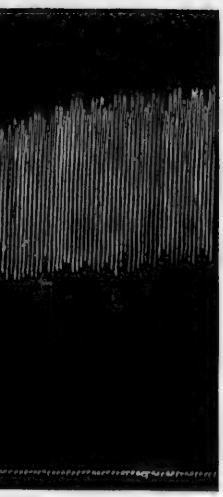


FIG. 10.

PLATE 35.



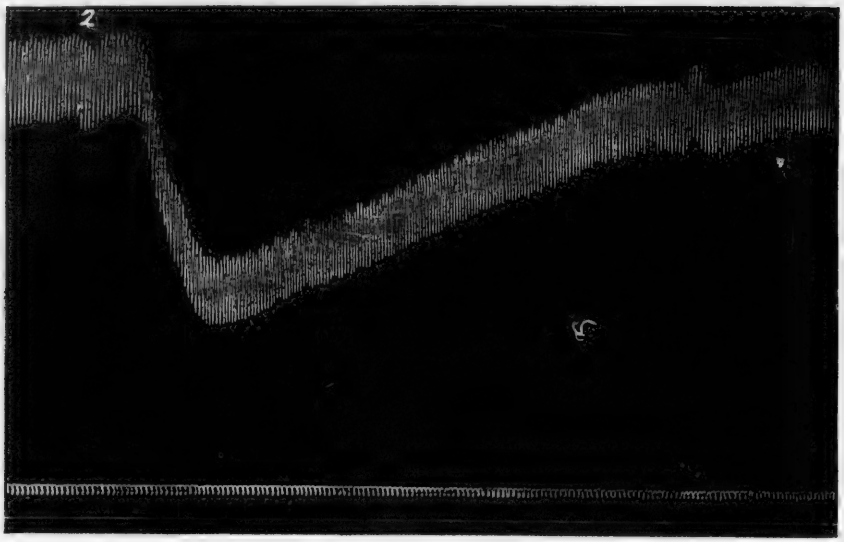


FIG. 11.

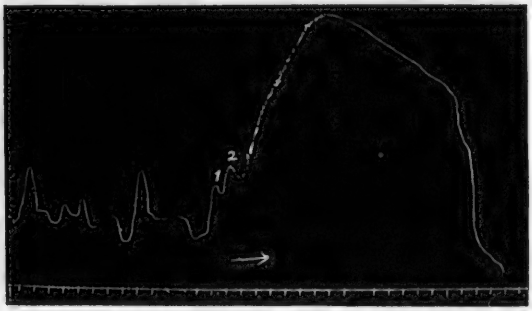


FIG. 15.

FIG. 18.

FIG. 12.

FIG. 13.

FIG. 14.

FIG. 16.

FIG. 17.

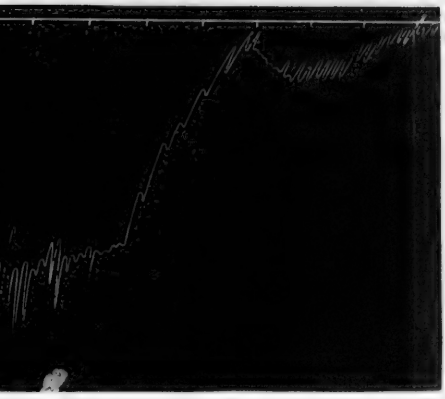


FIG. 19.

FIG. 20.

FIG. 21.

FIG. 23.

FIG. 25.

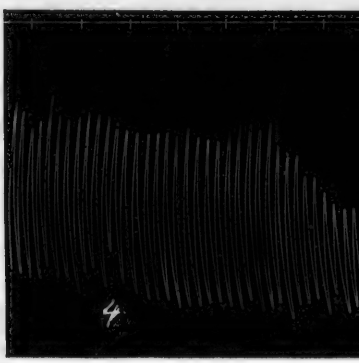


FIG. 26.

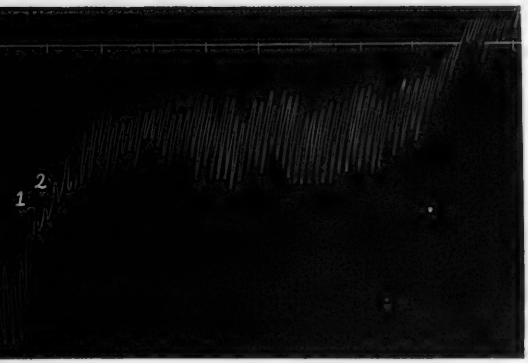


FIG. 22.

FIG. 24.

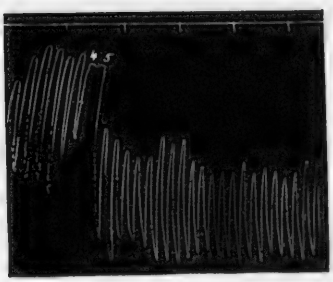


FIG. 27.

BILIARY CIRRHOSIS IN THE RABBIT.*

By MAURICE L. RICHARDSON.

(*From the Pathological Laboratory of the University of Wisconsin, Madison.*)

The statement concerning biliary cirrhosis which is commonly found in text-books on pathology is to the effect that obstruction of the bile-duct without infection does not lead to cirrhosis of the liver. This is expressed by Mallory,[1] in a recent article on cirrhosis, as follows: "Apparently uncomplicated bile stasis does not result in any sclerotic process around the hepatic veins where the chief lesion is located and it certainly does not around the portal vessels." This may be true for man; yet, if it be true, the reaction or lack of reaction in the liver would seem to form the only exception to the general rule that obstruction of the duct of a secreting gland leads to a (numerical) atrophy of the gland with secondary sclerosis of the organ. The liver in the rabbit forms no such exception. Ligation of the common bile-duct in the rabbit, without demonstrable succeeding infection, results in a cirrhosis of the liver.

The experiments here reported, which bear upon this assertion, are a portion of a series undertaken for the study of the regenerative phenomena occurring in the liver subsequent to injury of the organ. It was hoped to gain some information as to the factors which determine the variations in regenerative activity seen in different types of cirrhosis, in order to ascertain, if possible, why in some cases regeneration of the parenchyma is so marked a feature, while in others proliferation is apparently confined to the duct epithelium.

Among the procedures employed to injure the liver was ligation

*Received for publication, July 6, 1911.

[1] *Bull. Johns Hopkins Hosp.*, 1911, xxii, 69.

of the duct, and it is with the changes which follow such ligation that this paper chiefly concerns itself.

In performing these experiments, the rabbits have been placed under ether anesthesia, which has usually been of less than thirty minutes' duration. Under as nearly aseptic conditions as possible, the peritoneal cavity has been opened by a high median incision, and the common bile-duct located and ligated a short distance from its duodenal end, precaution being taken to avoid any manipulation of the liver which might lead to its injury. Double rows of sutures have been used in closing the abdominal wound. The lesion resulting has been studied at the end of twenty-four hour, forty-eight hour, one week, two week, and one month periods. All of the animals showed jaundice twenty-four hours after ligation, and in some cases it was very marked. The animals which were allowed to live beyond the first two days became progressively more and more emaciated, and at the end of two weeks they were almost devoid of subcutaneous or retroperitoneal fat. The animals seemed to have no inclination to eat for some time after the operation. Aside from jaundice and emaciation, there have been no post mortem changes which seem to need description in organs other than the liver, and, in consequence, general protocols of the autopsies are omitted.

Within twenty-four hours after the ligation of the bile-duct, well marked changes have taken place in the liver. In animals killed at that time, the gall-bladder and the bile-ducts are distinctly distended with clear, light green bile. The liver is definitely larger than normally and has a mottled appearance, opaque, light colored spots being set in the rather deeper brown general background. On section, there is a marked general cloudiness with a mottling due to the same opaque spots seen from the surface. These dots and areas of considerable size are scattered throughout the liver section. The lobulation of the liver is somewhat obscured.

The character of the change becomes apparent upon study of the microscopical sections. The liver cells generally are swollen and almost every cell has two nuclei, which is the result, as shown by study, of direct division of the nucleus. The most striking feature, however, is the presence of areas of necrotic cells, corresponding

to the opaque areas seen in the gross specimen. These areas apparently begin at the edge of the portal spaces and extend toward the central vein. In some places connective tissue of the portal space as well as cells of the lobules is involved; in other places it is not. The areas vary much in size and shape. They may include only six or eight cells in any one section, or they may involve one third of the lobule, and extend to the central vein. They tend, as a rule, to be oval in outline, but vary much from this usual shape. The necrosis appears to be of a peculiar type,—quite different from the hyaline necrosis so often seen in man. The cells stain very lightly with eosin, so that only their outlines are distinct. The nuclei either fail to stain or else appear as shrunken eosin-staining dots, while the protoplasm is represented by fine, faintly staining granules. At the edges of these areas, one finds in some places a sharp transition to more nearly normal cells, and in others, transition stages in which the first change appears to be a shrinkage of the nucleus leading to complete pyknosis. The cytoplasm in these transition stages also begins to stain less intensely. A Marchi stain for fat shows that in the larger areas of necrosis the liver cells toward the center are free from fat droplets, but that toward the periphery of the areas the capillaries and the capillary endothelium contain many fine and fairly coarse droplets.

There is in these areas of necrosis some invasion by leucocytes, which are found not only between the cells but also invading them. In some areas definite hemorrhages are found. In addition to these larger areas of necrosis, scattered necrotic cells may be found throughout the lobule. The portal spaces show dilatation of the bile-ducts and a spreading apart of the connective tissue fibrils by a fluid exudate. There is no leucocytic reaction about the ducts, nor are leucocytes found within them.

During the succeeding twenty-four hours there is an intensification of this picture, and some further important changes. The areas of necrosis are more numerous, their shape is more irregular, and they are larger. In some places the major part of a lobule is included in one area of necrosis. The hemorrhages into the areas are more numerous and more extensive. The individual cells of the larger necrotic areas show at this time no trace of a nucleus.

The shadowy particle seen at the end of twenty-four hours has disappeared. The outlines of the cells themselves have in many places become indistinct and even invisible, while the protoplasm of most of the cells is extremely tenuous, and appears to be undergoing rapid autolysis. In the central and, in greater numbers, in the mid-zone of the lobule, are scattered isolated cells showing either marked fatty degeneration or necrosis.

At this forty-eight hour period there is no sign of regenerative change in the parenchyma, as no mitoses could be found in the liver cells after very careful and protracted search. Conditions in the portal spaces, however, are quite different. Mitotic figures in the bile-duct epithelium are numerous, and bile-duct sprouts begin to appear. There is also even at this time an increase in cells of a fibroblastic character in the connective tissue of the spaces.

At the end of a week, there are gross signs in the liver of atrophy and scarring. The surface of the organ begins to have a slightly granular appearance, with slight depressions separating parenchymatous nodules. The liver is lighter in color and still shows a mottling with opaque, light yellowish dots. These are also evident on section. Upon microscopic study of the liver in this stage, areas of apparently recent necrosis are still found corresponding to those described for the earlier stages. Some of these areas are quite large and may even involve whole lobules and parts of adjacent lobules. Other areas of necrosis show complete disintegration of the cellular elements. These, as a rule, appear smaller than the recently necrosed areas and show in many places invasion by fibroblasts which have sprung from the tissue of the portal spaces. In some places, also, columns or cords of liver cells penetrate a short distance into the necrotic areas. Isolated necrotic liver cells are present throughout the lobule, as in earlier stages.

The portal spaces in this stage are much altered. The bile-ducts show a greater degree of proliferation, which is still in active progress, as is shown by the numerous mitotic figures in the duct cells. The bile-ducts radiate toward the edge of the liver lobules and show connection with and apparently differentiation into cells typical of the liver parenchyma. The connective tissue of the portal spaces has increased much in amount, and, as a result of its tendency to

invade the lobule in the areas of necrosis, and also at times to grow between apparently normal liver cells, the portal space begins to have a stellate appearance. In addition to the dilatation of the bile-ducts there is also a dilatation of the blood vessels of the portal space.

The picture at two weeks' time from the tying of the duct is practically an intensification of that after one week, as far as the growth of bile-ducts and of connective tissue is concerned. In one respect, however, it differs markedly. The larger areas of necrosis which formed so prominent a feature in the earlier stages are almost entirely lacking. Individual necrotic cells, however, are still found in abundance.

The last stage in the process studied was four weeks after the tying of the duct. At this period the bile-ducts are extremely dilated and tortuous. The common duct averages about seven millimeters in diameter. The gall-bladder is also much increased in size and tensely filled with clear green bile. It measures, roughly, five centimeters in length and between two and three centimeters in its widest part. The liver itself does not appear to vary much from the normal in size. It is light yellow in color and very granular in appearance. It is firm and cuts with difficulty,—the typical liver of an established cirrhosis. On microscopical examination, the most striking features of the liver are the smallness of the lobules and the increase in the portal spaces. With the removal of the necrotic cells, there has been a great reduction in the size of the lobule generally, as well as a distortion of the lobules in many places. The cells of the center of the lobule and, in fact, of all of the lobule save of the outer two or three rows, appear degenerated. Some are necrotic, others show fat vacuoles, while others merely stain more lightly and are loosely granular in appearance. The outer rows of cells have a much more normal appearance and seem to be continuous with the newly formed bile-ducts of the portal space. These latter are very numerous; indeed, the portal space appears to contain a thick network of the ducts. Where a distinct lumen is shown in the duct, bile pigment is often seen. The ducts branch toward the edges of the lobule and terminate in many places in cords of typical liver cells.

The connective tissue of the portal spaces is evidently still increasing. It still has a young appearance, and in no place has it the dense, scar-like appearance of old cirrhosis cases in man. The small parenchyma lobules are completely surrounded by connective tissue and are in many places invaded by fine strands which separate isolated liver cells and cords of cells.

In considering these experiments and the stages of the cirrhosis here described, in an attempt to determine the pathogenesis of the lesion, there seem to be few points of real difficulty, though some must still perhaps be left as debatable. The primary injury is very definite. It consists of scattered areas of necrosis of liver parenchyma, involving also capillaries and often some of the connective tissue of the portal spaces, beginning at the edge of the portal spaces and extending toward the central vein. There appears to be no doubt but that this primary lesion is due to the escape of the dammed back bile from the weakest point of the biliary conducting-system, *i. e.*, the juncture between the bile capillaries and the ducts proper. Whether this bile acts directly upon the liver cells or primarily upon the capillaries, producing a secondary anemic necrosis of the liver cells, is still questionable, though there are several features which indicate that both actions take place. The experiments of Dr. Bunting and Dr. Brown in this laboratory show that rabbit bile once elaborated is very toxic to rabbit tissues, even to the liver when applied to it externally. It will also cause necrosis of vessel walls and thus thrombosis. The lack of any relation of some of the irregular necrotic areas to blood supply and blood flow, and the occurrence of small areas consisting of a very few cells and even of individual cells, might indicate direct action. On the other hand, in some cases definite thrombi were found in the portal vessels. Furthermore, the occurrence of dilatation of portal vessels in later stages would indicate interference with the circulation within the lobules, as it occurs before there is sufficient connective tissue growth in the spaces to impede flow. In this primary injury one has all the elements that Mallory considers necessary for the production of a cirrhosis,—injury to parenchyma, to connective tissue, and to blood vessels; there might be added tension of the tissues due to dilatation of the ducts.

In the reaction to this injury, the bile-duct proliferation is the most marked early feature, though the connective tissue growth begins at the same time. The immediate stimulus to the bile-duct epithelium would appear to be the increased tension due to the damming back of bile. That there is a very active proliferation is shown by the large number of mitotic figures found early and even after two weeks' time. In fact, bile-duct proliferation appears to be the chief regenerative feature on the part of the epithelial elements. I am not inclined to favor the term "pseudo-bile-ducts" for the resultant growths. As MacCallum[2] has shown, in acute yellow atrophy in man, the proliferating bile-ducts show a distal differentiation into typical liver cells. In man, this attempt at regeneration appears to be abortive. In these experiments in the rabbit, however, it appears less so. The distal ends of the ducts become differentiated into cords of liver cells in some places; in others, they seem to unite directly with the existent cells of the lobule. Their lumina contain bile. In the latest stage studied, regeneration from the bile-ducts seemed to be responsible for the more normal liver cells forming the periphery at the lobule. Regeneration on the part of the parenchyma, however, appears in these experiments to have been very slight. No mitoses were found in parenchyma cells at any stage, though they were carefully searched for. That there was some regeneration the sections show definitely, in the invasion of necrotic areas by columns of liver cells, but it falls far short of that seen after a single toxic injury, and the gradual shrinkage in the size of the liver lobules would seem to indicate that the disturbance in function of the cell and the damming back of the toxic bile were handicaps too great for the parenchymatous cells to overcome. The proliferation of connective tissue elements was prompt and progressive throughout the period studied, mitoses being still present in the one month stage. Finally, in no case was any microscopical evidence of infection of the bile-ducts found.

In conclusion, I desire to thank Dr. Bunting for the interest he has taken and for the assistance he has given throughout the course of this study.

[2] *Johns Hopkins Hosp. Rep.*, 1902, x, 375.

ON THE RELATION OF THE OPTIC THALAMUS TO RESPIRATION, CIRCULATION, TEMPERATURE, AND THE SPLEEN.*

By ERNEST SACHS, M.D.

(*From the Department of Physiological Chemistry, Cornell University, New York.*)

PLATES 38–43.

SYNOPSIS

HISTORICAL.
1. Circulation.
2. Respiration.
3. Spleen.
4. Temperature.
DESIGN OF RESEARCH.
METHOD AND TECHNIQUE.

STIMULATION EXPERIMENTS ON THALAMUS AND ADJACENT REGIONS.

Group I. Circulation.
Respiration.
Spleen.
Group II. Temperature.
DISCUSSION AND CONCLUSIONS.

In the year 1909, I published in *Brain*[101] an article recording the results of a series of experiments on the optic thalamus in which its anatomy and some of its functions were considered. That investigation was carried on by a more accurate method than had hitherto been employed; namely, with Clarke's stereotaxic instrument. This method proved so useful that a further study of certain functions of the thalamus was undertaken. In the present paper, I have considered four points which have been under discussion for some time; namely, the relation of the thalamus to circulation, respiration, spleen, and temperature.

*Received for publication, June 30, 1911.

HISTORICAL.

Since my previous communication, a few contributions have been made to the subject. D'Abundo's[2] experimental study has not added anything very new to the subject. His lesions were large and yielded for the most part the same results as Roussy[68] obtained. He has made no observations on the questions that interest us here. The valuable paper of Malone[74] on the nuclei of the thalamus divides the thalamus, by the Nissl method, into such a large series of nuclei that we cannot use his classification for our studies. The papers of Aisenstat,[3] Sinelnikow,[107] and Streerath,[110] on the heat centers in the thalamus and corpus striatum, we shall speak of under the heading of temperature.

(1) *Circulation.*—Since Danilewsky[20] first claimed that certain regions of the thalamus and caudate nucleus were concerned with changes in blood pressure and pulse rate, a number of other observers have directed their investigations towards this subject. Simbriger,[106] Hitzig,[53] Balogh,[9 10] Howell and Austin,[55] Prus,[92] and Bechterew and Mislawsky,[12 13] all found such changes, a rise of blood pressure and at times a change in rate. Carville and Duret,[24] Beevor and Horsley,[15] and some others obtained absolutely negative results.

The most careful work on the positive side was that of Prus, whose table shows different effects on the thalamus according to the point stimulated. However, no observer, not even Prus,[92] has attempted to correlate the points of stimulation with the various anatomical regions of the thalamus. This is due to the large lesions and inaccurate methods used. Opinions also vary somewhat as to the cause of these phenomena. Thus Bechterew and his school claim that these points stimulated are circulatory centers. Such a conclusion is not warranted, for we shall see later on that these changes are very similar to those observed when a peripheral sensory nerve is stimulated. The view advanced by Howell and Austin in their brief communication seems more nearly in accordance with the facts. Tigerstedt[113] and Boruttau[20] hold a very similar point of view; namely, that such phenomena may be simply reflex.

(2) *Respiration.*—Few chapters in physiology have a more extensive or confusing literature, and as recently as 1909, Boruttau[24] declared that the views on the nervous control of respiration were so varied that many experiments were still necessary to clear it up. A general survey of the subject leads inevitably to the conclusion that investigators have been but too ready to claim the existence of a respiratory center in any region from which, upon stimulation, respiratory phenomena were elicited. Marckwald,[78] Rosenthal,[96] Christiani,[25 26] Martin and Booker,[77] Asher and Lüscher,[7] and Lewandowsky[65] are some of those who have found respiratory changes upon thalamic stimulation. Martin and Booker[77] described four kinds of changes: inspiratory and expiratory inhibition, rapid respiration, and inspiratory tetanus.

The mechanism involved in these changes has received innumerable explanations which cannot, for lack of space, be considered here. Boruttau has reviewed the subject clearly and objectively. The experiments were carried on sometimes by stimulation of the basal ganglia after extirpation of the cortex, and then again by introducing electrodes or other instruments directly into the regions to be studied. Besides considering the region stimulated, a number of factors have been mentioned from time to time that were supposed to influence

respiratory movements. The most important of these were the type of stimulus, whether chemical, mechanical, or electrical (Eckhard,[32] Budge, Bernard,[17] Rosenthal[97]), the sex of the animal (Meltzer),[78] and other even more unusual factors. Subsequently the views of von Helmholtz[47] and a few other investigators gained ground; namely, that the character and strength of the stimulus were the important factors. Lewandowsky[66] went so far as to claim that weak tetanizing currents produced inhibition of respiration, stronger currents rapid respirations, and spreading currents inspiratory tetanus, and that this latter stimulus was comparable to the painful stimulus of a sensory nerve. Boruttau[21] claims that the length of stimulus determines whether there shall be acceleration or inhibition of respiration. This has been strenuously denied by others, and the present investigation, as may be seen from a glance at the records, does not support such a view. From our records it is evident that acceleration or inhibition starts at the moment the stimulus begins, regardless of its length. Spencer,[109] after repeating Christiani's experiments, does not see why, if respiratory centers are assumed, as such, excitation experiments are better explained by attributing their results to stimulation of fibers rather than to stimulation of central mechanisms. In summing up this chapter, Boruttau[84] says: "It seems most probable that all cerebral and spinal tracts of general sensibility, particularly those of the respiratory paths and respiratory muscles, are connected directly with the respiratory center tentatively assumed to lie in the region of the corpora quadrigemina."

(3) *Spleen.*—Erikson, working in Bechterew's laboratory, as reported in Bechterew's book on the nervous system, maintained that there was a center in the thalamus controlling movements of the spleen. In Bechterew's book (vol. 2, pp. 1166 and 1167) are some of the kymograph records. These are so inaccurate and show such mediocre work that that alone makes us doubt his results. Furthermore, one single experiment, in which when there was a movement of the spleen the animal also urinated, is cited as proving that the bladder and splenic centers lie near each other in the thalamus. Then, too, we find no mention in his text of the normal splenic wave (figures 1, 3, and 6) first described by Roy,[99] and later more exhaustively studied by Schäfer and Moore.[103] Some of Bechterew's records look strikingly like these normal splenic waves. These observations of Erikson on the relation of the thalamus to the spleen have never been confirmed. Roy[99] says that the drop in the specific rhythm synchronous with the rise in blood pressure is a vaso-constrictor action arising in the medulla.

(4) *Temperature.*—It has been claimed that the thalamus and corpus striatum control temperature. Lesions of these regions are said to cause a rise of temperature. Aronsohn and Sachs,[6] Ott,[87–90] White,[118] Richet,[93] Tangl,[112] Ito,[56] and many others have made such observations. Most of these studies have been on rabbits, some on dogs, and Tangl's four experiments were on horses. White,[118] though first inclined to think that the thalamus was concerned with these changes in temperature, in a later publication ascribes them all to the corpus striatum. He also observed clinically marked rises of temperature, and such observations are not uncommon in apoplexies with ventricular hemorrhage. Mosso[80–83] is the chief opponent to this view and in a series of investigations has pointed out many of the errors in connection with it. Thus he found that the temperature of rabbits

varies greatly (1° to 2° C.) under normal conditions without any operative procedure at all. Some of the possible causes postulated by him for the rise of temperature are hemorrhage and excitement. Muscular contractions may cause a rise, which some investigators have excluded by the use of curare. Under such conditions Aronsohn[5] found no rise of temperature. Such experiments as the four of Tangl on horses are quite inconclusive, for only two showed a post-operative rise, and in these the author does not say how much hemorrhage occurred. A large experience in surgical wards has shown me repeated examples of post-operative rises of temperature in cases where there was no hemorrhage, no traumatism of tissues, and in which the wounds healed *per primum* without any reaction. This makes one very skeptical as to the cause of any particular post-operative rise of temperature. These criticisms hold also in regard to the three researches published in the past year by Aisenstat,[3] Sinelnikow,[107] and Streerath.[110] The latter, though he says that the rabbit's temperature may vary 1.3° C., always makes his calculations from the minimum temperature.

Tigerstedt[113] sums up the subject well when he says that there is little evidence of a temperature center in the brain, though there is some evidence that there may be one in the corpus striatum.

DESIGN OF THE PRESENT RESEARCH.

From this brief review, it is apparent that it has not been established what, if any, is the relation of the thalamus to circulation, respiration, the spleen, and temperature. The more accurate method at my disposal has enabled me to throw some further light on these questions and to place the true functions of the thalamus on a firmer basis. In the course of this work, I have made some observations on other portions of the brain and these will also be reported.

METHOD AND TECHNIQUE.

In the present investigation, ninety-two cats were used. Only sixty of these are made use of in the present report. The thirty-two not incorporated were discarded either because they died before the termination of the experiment—usually from an overdose of ether—or because the records were not clear enough to study. With the exception of one or two, all of these were animals employed in the earlier experiments, when we were perfecting the methods of procedure. The experiments will be reported in two groups.

I. Electrical stimulation of the thalamus and neighborhood with reference to circulation, respiration, and the spleen.

II. Electrical stimulation of the thalamus, caudate nucleus, and lenticular nucleus with regard to temperature.

The animals were all operated upon under ether anesthesia. Aseptic methods were not used, as each animal was killed at the end of the experiment, before recovering from the anesthetic. The head with the brain *in situ* was hardened in 10 per cent. formalin, and after several days the brain was removed, cut on a macrotome to show the lines of stimulation, photographed (figures 8 to 12), and then cut in serial sections and stained with polychrome methylene blue in order to identify more accurately the region that had been stimulated.

The Clarke instrument is fully described by Horsley and Clarke[54] in their account of experiments on the cerebellum. Bipolar stimulation was used throughout with needles measuring 1 to 1.5 millimeters in diameter. The needles, as seen from the course of the needle track in the photographs, were introduced through the vertex (figures 8 to 12). Bipolar stimulation was used, as the unipolar method with one electrode in the brain substance and the other on the surface yields unreliable results, as pointed out in Horsley and Clarke's[54] article and in my own.[101] The current was obtained from an induction coil which was supplied by one dry cell. A minimal current was used at all times. At no time was this current more than would yield a moderate response if applied to the motor cortex. If at any time such a current was too powerful, it was reduced until a point was reached where the weakest possible current yielded a result. During the experiments the brain was kept warm by constant irrigation with warm solution, and only as long as the animal showed no fatigue was an experiment continued.

Circulation was studied by means of a cannula in the carotid, connected with a mercury manometer. For recording the respiration, a cannula with three arms was inserted into the trachea; one of these arms was connected with an ether bottle, one was a safety opening to admit air rapidly in case of a collapse, and the third was connected with a large bottle which in turn was connected with a modified Brodie receiving tambour made of a large, thin-walled ice-bag. By this device the upstroke on the records represents

expiration, and the downstroke, inspiration. The large bottle was inserted to lessen the depth of excursion of the lever. The arm leading to the ether bottle had a three-way stopcock so that pure air, pure ether, or a mixture of the two could be given. When a kymograph record was taken, all connection with the outer air was excluded, and for this purpose the large bottle was also useful, as it contained enough air to prevent asphyxial symptoms. Between each two stimulations the animal was given fresh air.

Splenic records were obtained with an oncometer of the type described by Schäfer and Moore,[103] with air transmission. The receiving tambour was a modified Brodie tambour made of part of a small finger cot. In these records the upstroke represents expansion of the spleen body. In the temperature experiments, the animal was kept on a warm stage. The temperature was taken per rectum with a Heidenhain thermometer made by Koehler and Martini in Berlin.

In localizing any point in the brain when using the Clarke instrument, the brain is conceived as divided by three planes: (1) The sagittal plane passes longitudinally between the two hemispheres. The plane sections parallel to it are spoken of as lamellae 1, 2, 3, according to their distance in millimeters from the sagittal (zero) plane. (2) The basal plane has its four points determined by the inferior margin of the bony orbits and the centers of the bony auditory meati. The horizontal plane employed for convenience, as zero, is parallel to this and ten millimeters higher in the average cat. The height above or below this is designated in letters thus: A = one millimeter above the plane; —A = one millimeter below the plane; B = two millimeters above, etc. (3) The frontal or interaural plane, perpendicular to the horizontal plane, passes through the centers of the two meati. The numbers 1, 2, 3, etc., designate the distance in millimeters in front of or behind this plane.

In the kymograph records, each point of stimulation has been lettered according to this nomenclature. This, however, need not concern the reader, since he is only concerned in knowing what anatomical region was stimulated, and as animals' heads vary somewhat. Lamella 2, B 10, for example, would not correspond to the same point in every brain; but knowing that a certain result was

obtained there, one could readily identify the spot on the brain sections as it would be two millimeters from the median line, two millimeters above the horizontal plane, and ten millimeters in front of the interaural plane. In each experiment, stimulation was carried on in but one lamella, but not every point in the thalamus in that lamella was stimulated in each experiment. Therefore I have selected type photographs of each lamella. These show what points were stimulated during the various experiments. From these and the tables which record the regions stimulated in each experiment, it will be seen that the thalamic nuclei were repeatedly stimulated as well as certain neighboring regions. In these experiments I have adopted the same classification of the thalamic nuclei which I used in my previous paper,[101] as it is most practical for recording these experiments, as follows: nucleus anterior, nucleus medius, nucleus reuniens, nucleus lateralis, nucleus ventralis, center median and nucleus arcuatus, and pulvinar.

The results can best be stated by taking up each nucleus separately and then the adjacent regions.

GROUP I.

Nucleus anterior.—(Table II.) In seventeen cats the anterior nucleus was stimulated. In all but four instances there was a respiratory change, usually of an expiratory inhibitory character. These inhibitory phenomena usually consisted of a moderate slowing of the rate. There were four instances of inspiratory inhibition, —a very unusual phenomenon in the thalamus. So unusual is this occurrence, and so rarely was it observed in any other region (see experiments 141, 145, 191, 193, table I) of the thalamus, that we consider it the exception.

A rise of blood pressure was the rule, and in proportion as the blood pressure rose the spleen changed in volume; that is, where there was a slight rise no change occurred, but when there was a rise of more than five millimeters the spleen showed a change in volume, and this at times was an increase, at other times a decrease. There seemed no way of predicting what the nature of this change would be; sometimes the pressor effect predominated, and then

again the depressor. The volume changes in the spleen are influenced entirely by the blood pressure changes. We have never observed a change in the spleen without an associated change in the blood pressure. No change of impulse rate was observed at any time. In our previous communication, we pointed out a close relation between the anterior nucleus and the nucleus caudatus. The anterior nucleus was as unresponsive as regards motor phenomena as was the caudate, and, furthermore, we traced degenerations running from it along the stratum subcaudatum. The present facts clearly show that though neither is concerned with motor phenomena, stimulation of the anterior nucleus affects respiration and blood pressure, while the caudate is inert to stimulation.

Nucleus medius.—(Tables I and II, figure 3, C 10, O 10.) In thirteen experiments the results were uniform. No respiratory or circulatory changes were observed. This nucleus is then as inert in respect to these functions as it is in respect to the motor phenomena. It is of particular interest to note that this applies only to the dorsal part of the nucleus and that in this region there are no fibers from the superior cerebellar peduncle, while in the ventral portion, occupied by the nucleus reuniens, the fibers of the superior cerebellar peduncle are found (Horsley and Clarke).

Nucleus reuniens.—(Table II, figure 3, —B 10; figure 4, —B 8.) In ten experiments the marked difference between the dorsal and ventral portions of the nucleus medius was apparent. This consisted of total inspiratory inhibition following stimulation, with a condition of inspiratory tetany (Martin and Booker),[77] and this was associated with forced inspiration. The blood pressure rose markedly, though the pulse rate remained the same. The changes in the spleen were only observed when the blood pressure rose a great deal, and at such times the progression movements, so characteristic of the superior cerebellar peduncle, contributed to the apparent volume increase of the spleen. This accounts for the very unusual rise in figure 3, —B 10. That the nucleus reuniens, which was first described by Ziehen[122] in the marsupials, should always have been considered a part of the nucleus medius, is amply accounted for by the physiology of the former, as well as by its anatomy.

Malone[74] has but recently pointed out how very different its structure is from the rest of the nucleus medius.*

The type of respiration here observed is similar to that seen on stimulation of a more posterior part of the thalamus and of the region about the corpora quadrigemina. The posterior region is the one in which Christiani[25, 26] placed his respiratory center, yet here we observe the same phenomena in connection with a region much more anterior.

Nucleus lateralis.—(Table I, figure 1, C 12, O 12.) The twenty observations all show a respiratory change which is more marked in connection with the ventral and posterior portions of the nucleus. The excitation phenomena increase in intensity in proportion as there are more fibers from the superior cerebellar peduncle and median fillet. In my previous paper, already referred to, it is pointed out that the largest number of fibers from these tracts end in the ventral and posterior portions, while they become fewer in the anterior and dorsal portions. About half of the observations show a change in blood pressure, while the others show none. In those in which the change occurred, the region stimulated was this same more ventral, more sensitive portion. The pulse rate remained the same throughout and the spleen was unaffected.

Nucleus lateralis ventralis.—(Tables I and II, figure 1, —B 12.) Here in fifteen observations the same conditions were observed as were noted in connection with the nucleus lateralis, but here they occurred with more intensity. The anatomic connections of the nucleus lateralis ventralis with the sensory paths, superior peduncle, and fillet, are the same as those of the nucleus lateralis. It is of interest to note that as we approach the base of the nucleus lateralis ventralis,—the hypothalamic region (figure 4, —B 8),—the respiratory phenomena change and inspiration is differently affected, which we shall see later is characteristic of the globus pallidus of the nucleus lenticularis.

Pulvinar.—(Tables I and II.) Seventeen observations indicate greater excitability of the median portion than the lateral portion, and this becomes more marked as the posterior commissure is

* Edinger[33] pointed this out some years ago in the invertebrates, and I[100] identified it as a separate structure in the vertebrate series.

approached. The respiration on stimulation of this region shows at times expiratory inhibition or some acceleration, and in this respect it is of the same type as in the rest of the thalamus. The blood pressure changes are less marked, but still present. The median portion of the pulvinar is evidently more sensitive on account of its more intimate connection with the fillet (Probst) and with the central grey and nucleus fasciculis longitudinalis posterior (Edinger).[33]

Posterior Commissure.—(Tables I and II.) Nine observations were made. In this region, as already stated under the last heading, the excitability is greater, and shows itself by inspiratory tetanus, and by a rise of blood pressure which at times is so marked that the spleen volume changes. The character of the respiratory change is identical with that observed upon stimulation of the nucleus reuniens (figure 3, B 10—B 10).

Ganglion Habenulae and Meynert's Bundle.—(Table II.) The ten observations are uniform in their results and practically identical with the findings in the active parts of the thalamus. This, the epithalamus, Edinger[33] claims is connected through the taenia thalami with the general olfactory system. That this should be so very excitable is not surprising since this system is highly developed in the carnivora.

Fornix.—(Tables I and II, figure 5, C 4.) Thirteen observations were made. This region has invariably proved inert to stimulation even with strong currents. In the record shown, the cornu ammonis of the fornix was stimulated, but the same negative result was also obtained from stimulation in other parts of the fornix, as is shown in the photographs.

Fibers of Lancisi.—(Table II, figure 1, G 12.) Nine observations were recorded. This region invariably yielded no result on stimulation. It is of interest and perhaps somewhat surprising that such marked effects should be obtained from one part of the olfactory system—the ganglion habenulae—while the fornix and fibers of Lancisi were inert. This, however, seems readily enough explained by the fact that the latter two belong to a higher system of neurones and do not take part in a lower reflex mechanism.

Fillet.—(Table I, figure 5, O 4.) Six observations were made.

Here, too, we observe the same respiratory phenomena and inspiratory tetanus, which we have observed both in the nucleus reuniens and the ventral portion of the lateral nucleus. This was to be expected, for here we have but stimulated in their course the fibers whose terminations we stimulated in the nucleus reuniens, nucleus lateralis, and nucleus lateralis ventralis.

Central Grey.—(Tables I and II, figure 6, O 3, —A 3, —B 3.) Twenty observations were made. This region yields on stimulation very marked results; it seems that of all the regions stimulated, respiratory changes are most pronounced in this region. The results on stimulation were always very rapid respiration and what Martin and Booker[77] have described as inspiratory tetanus. Together with this the blood pressure rose, and at times, when marked blood pressure change occurred, the volume of the spleen was affected. The pulse rate never changed. This region is unquestionably the one that Christiani[25] described as a respiratory center. The central grey substance, however, as pointed out by Edinger,[33] and Meynert and Obersteiner, runs from the thalamus down to the fourth ventricle and contains fibers which connect all the cranial nerves with the thalamic region. The chief one of these tracts and the one that concerns us especially here is the dorsal longitudinal bundle of Schütz,[105] which would carry any impulse directly to the vagus nucleus.

A few isolated observations recorded on tables I and II of stimulation of the anterior and posterior corpora quadrigemina (figure 7, O 4, —A 4, —B 4), Türck's bundle, and descending root of the fifth nerve, only serve to show that from these regions also respiratory and blood pressure changes may be obtained.

Nucleus caudatus.—(Tables I and II.) Of twelve observations made in this region all were negative, and even with strong currents no result was obtained. This confirms what the most careful observers have repeatedly maintained, that the nucleus caudatus is inert to stimulation as regards any changes in respiration or circulation.

Nucleus lenticularis (*Globus pallidus*).—(Tables I and II, figure 2, C 15, O 15.) Eleven observations. By stimulation here we invariably obtained inspiratory inhibition with slowing of respira-

tion, but at no time was there any similarity to the inspiratory phenomena noted on stimulation of the central grey. In the latter case there was forced inspiration followed by tetanus, while in stimulation of the globus pallidus complete inspiration was stopped and the lungs were never filled to their full normal capacity. In some of these observations, blood pressure changes were also noted, but we are inclined to think that pure globus pallidus excitations are unaccompanied by blood pressure changes, since in those cases in which the blood pressure was affected, our electrodes were at the innermost edge of the globus pallidus, next to the internal capsule and nucleus lateralis ventralis.

GROUP II.

Temperature.—The experiments on this important phase of the subject number eight, of which one is a control.

They were carried on differently from those generally reported in the literature, which we have reviewed in the beginning of this paper. Before a final verdict can be rendered, other experiments must be performed. We feel, however, that these observations clear up one phase of the subject absolutely.

If a certain region of the brain is a true temperature center, and if the nerve paths which pass out of it have that specific function, stimulation with a faradic current ought to yield some change in temperature. Furthermore, such a change ought to occur as promptly as the result following any excitation of a nerve or nerve center. If the lesions made in the various experiments of other observers are to be construed as destructive ones, electrical stimulation of such a center ought to produce the opposite effects. If those lesions act as mechanical stimuli, faradic excitation ought to produce the same result. By not allowing the animals to live for several days, as previous investigators have done, we have excluded a large number of possible sources of error which were enumerated in the historical review. I wish to emphasize here once more that with the most painstaking surgical technique, where no blood has been lost, and where no infection and no traumatism of tissues have occurred the day following operation, a post-operative temperature rise of several degrees occurs. In reviewing the experiments in the

literature, I have attempted to discover whether in those experiments in which excessive rises in temperature occur there was much bleeding from the diploic vessels, and whether the experimenters used bone wax, the quickest and most effectual method of stopping such hemorrhage. This is of particular importance in experiments on carnivora, as their diploic vessels are very well developed. The experiments reported, however, throw no light on this question at all.

From the appended tables it is evident that all parts of the thalamus, nucleus caudatus, and nucleus lenticularis were repeatedly stimulated. Never was the slightest effect noticed upon the temperature. All the animals, like control cat 105, showed a gradual progressive fall of temperature which in the course of an hour amounted to from 1° to 2° C. This gradual fall was independent of the stimulation, for in every instance the temperature recorded in the tables was the same before and after each stimulation. The gradual fall occurred between stimulations.

Cat 97. April 16, 1910. Lamella 2.

Temperature before operation, 37.6° C.
Temperature after operation, 37° C.
Each stimulation lasted 30 seconds unless specially indicated.

Point of stimulation.	Temperature.	Region.
*J 15	36.5	Cingulum
E 15	36.5	Fornix
O 15	36.45	Nucleus anterior
C 15	36.45	Nucleus anterior
1 min.—B 15	36.5	Nucleus lateralis (muscular movements)
J 10	36.4	Fibers of Lancisi
E 10	36.4	Fornix
C 10	36.35	Ganglion habenulae
1 min. O 10	36.4	Meynert's bundle (respirations violent, current strong)
—B 10	36.3	Nucleus reuniens
E 6	36.25	Corpus callosum
C 6	36.2	Anterior corpus quadrigeminum
1 min. O 6	36.2	Central grey
1 min.—B 6	36.15	Central grey
E 3	36.2	Ventricle
C 3	36.1	Central grey
2 min. O 3	36.1	Central grey
1 min.—B 3	35.95–35.8	Posterior longitudinal bundle

*For my abbreviations, see under "Method and Technique."

Cat 101. April 20, 1910. Lamella 4.

Temperature before operation, 37.45° C.
Temperature after operation, 37.15° to 36.6° C.
Each stimulation lasted one minute unless otherwise indicated.

Time.	Point of stimulation.	Temperature.	Region.	
30 seconds	E 14	36.5	Anterior nucleus	
30 seconds	C 14	36.4	Lateral nucleus	
	O 14	36.4	Lateral nucleus	
	—B 14	36.35	Globus pallidus	
	E 10	36.3 to 36.2	Lateral nucleus	
	C 10	36.2	Median nucleus	
	O 10	36.15 to 36.1	Median nucleus	
	E 6	36.0	Ventricle	
	C 6	35.95	Anterior corpus quadrigeminum	
30 seconds	O 6	35.9	Central grey	Respirations stopped
10 seconds	—B 6	35.65	Central grey	Respirations stopped
	E 3	35.5	Ventricle	
	C 3	35.45	Posterior corpus quadrigeminum	
	O 3	35.45	Central grey	
	—B 3	35.4	Central grey	Violent muscular contractions

Cat 103. April 22, 1910. Lamella 6.

Temperature before operation, 37.75° C.
Temperature after operation, 37.2° C.
Each stimulation lasted one minute.

Point of stimulation.	Temperature.	Region.	
E 14	37.1	Nucleus caudatus	
C 14	37.05	Nucleus caudatus	
O 14	36.75	Internal capsule	
—B 14	36.7	Globus pallidus	
E 10	36.65	Nucleus lateralis	
C 10	36.6	Nucleus lateralis	
O 10	36.55	Nucleus lateralis ventralis	Violent muscular movements
—B 10	36.5		
E 6	36.5 to 36.4	Ventricle	
C 6	36.35	Pulvinar	
O 6	36.3	Pulvinar	
—B 6	36.25	Median fillet	
E 3	36.2	Ventricle	
C 3	36.1	Posterior corpus quadrigeminum	
O 3	36.05	Fillet	
—B 3	36.0	Fillet	Muscular movements
—D 3	36.0	Türck's bundle	

Cat 105. April 25, 1910. Control Experiment.

Temperature before operation, 37.7° C.

12.20. Temperature after operation, 37.4° C.

This animal was operated upon, as were all the others, by a craniotomy with opening of the dura. It was kept alive as long as the duration of the average experiment on the thalamus. No lesions were made, but every other step was taken.

Time.	Temperature.	
12.25	37.35° C.	
12.30	37.1° C.	
12.40	36.85° C.	
12.45	36.6° C.	
12.50	36.4° C.	
12.58	36.15° C.	Experiment stopped

Cat 107. May 4, 1910. Lamella 8.

Temperature before operation, 37.95° C.
Temperature immediately after operation, 37.65° C.
Temperature 20 minutes later, 37° C.
Each stimulation lasted one minute.

Point of stimulation.	Temperature.	Region.
E 14	36.9° C.	Internal capsule
C 14	36.8° C.	
O 14	36.75° C.	Putamen
—B 14	36.7° C.	
E 12	36.6° C.	Nucleus lateralis
C 12	36.6° C	
O 12	36.5° C.	Nucleus lateralis ventralis
—B 12	36.45° C.	
E 10	36.4° C.	Nucleus lateralis
C 10	36.3° C.	
O 10	36.2° C.	Nucleus lateralis ventralis
—B 10	36.1° C.	Hypothalamus
E 8	36.05° C.	Fornix
C 8	36.° C.	
O 8	35.95° C.	Corpus geniculatum exterior
—B 8	35.9° C.	
E 6	35.75° C.	Pulvinar
C 6	35.7° C.	
O 6	35.65° C.	Corpus geniculatum exterior
—B 6	35.6° C.	
E 3	35.5° C.	Ventricle
C 3	35.5° C.	
O 3	35.4° C.	Corpus quadrigeminum and pulvinar
—B 3	35.3° C.	

Cat 199. May 12, 1911. Lamella 9.

1.30 P. M. Temperature before operation, 38.9° C.
1.40 P. M. Temperature after operation, 38.27° C.
Each stimulation lasted one minute.

Point of stimulation.	Temperature.	Region.
E 16	38.7° C.	Internal capsule
C 16	38.65° C.	
A 16	38.6° C.	Putamen
—A 16	38.6° C.	
E 13	38.6° C.	Internal capsule
C 13	38.6° C.	
A 13	38.55° C.	Putamen
—A 13	38.55° C.	
E 10	38.55° C.	Pulvinar
C 10	38.5° C.	
A 10	38.45° C.	Geniculate lateral
—A 10	38.45° C.	
E 7	38.4° C.	Pulvinar
C 7	38.4° C.	
A 7	38.4° C.	Cornu ammonis
—A 7	38.35° C.	
—C 7	38.3° C.	

Experiment ended at 2.45 P. M. (same day).

Cat 201. May 26, 1911. Lamella 7.

10.40 A. M. Temperature before operation, 37.7° C.
10.50 A. M. Temperature after operation, 37.6° C.
11.00 A. M. Temperature after operation, 37.8° C.
Each stimulation lasted one minute.

	Point of stimulation.	Temperature.	Region.
	E 15	37.6° C.	Nucleus caudatus
	C 15	37.5° C.	
	A 15	37.5° C.	Internal capsule
	—A 15	37.5° C.	
11.20	E 12	37.4° C.	Internal capsule
	C 12	37.35° C.	
	A 12	37.3° C.	Lateral nucleus
	—A 12	37.25° C.	
	E 9	37.2° C.	Lateral nucleus
	C 9	37.15° C.	
	A 9	37.1° C.	Lateral ventral nucleus
	—A 9	37.05° C.	
	E 5	37.0° C.	Cornu ammonis
	C 5	37.0° C.	
	A 5	36.95° C.	Pulvinar
	—A 5	36.925° C.	
11.45		36.8° C.	
12.05	Experiment ended	36.3° C.	

Cat 203. June 1, 1911. Lamella 5.

10.50 A. M. Temperature before operation, 37.8° C.
11.00 A. M. Temperature after operation, 38.0° C.
11.15 A. M. Temperature after operation, 37.2° C.
Each stimulation one minute unless otherwise mentioned.

Point of stimulation.		Temperature.	Region.
30 seconds	E 15	37.05° C.	Nucleus caudatus
	C 15	37.0° C.	
	A 15	37.0° C.	Internal capsule
	—A 15	37.0° C.	
	E 12	36.95° C.	Nucleus anterior
	C 12	36.85° C.	
	A 12	36.75° C.	Median nucleus
	—A 12	36.7° C.	
	E 8	36.65° C.	Pulvinar
	C 8	36.6° C.	
30 seconds	A 8	36.65° C.	Nucleus lateralis (violent muscular movements)
	—A 8	36.65° C.	
	E 4	36.5° C.	Anterior corpus quadrigeminum
	C 4	36.4° C.	
	A 4	36.4° C.	Commissure of anterior corpus quadrigeminum (violent muscular movements)
	—A 4	36.3° C.	

Experiment lasted about one hour.

DISCUSSION AND SUMMARY.

From what has already been said, it is apparent that changes in respiration and blood pressure may be produced by stimulation of a very large portion of the optic thalamus; that no one region controls these phenomena; that the spleen shows variations in volume only when marked blood pressure changes occur, and that such a change has been observed on stimulation of many places in the thalamus. Furthermore, the regions in the thalamus from which such effects are obtained are those in which the large afferent paths end; namely, the fillet and superior cerebellar peduncle, and a region directly connected with the vagus nucleus. The portions of the thalamus which proved inert to stimulation were those which in our previous investigation also were inactive; namely, the median nucleus, and the upper anterior end of the lateral—the region through which the fibers of the median nucleus pass.

These phenomena are similar in every respect to those observed when an afferent nerve is stimulated. In what has gone before, it has been shown that the respiratory and circulatory changes occurred when one of the sensory paths, or a path running directly to the medullary nuclei governing these functions was stimulated. In explaining these phenomena, therefore, there is no necessity for invoking the existence of special centers.

CONCLUSIONS.

1. There is no center controlling respiration in the optic thalamus, but any changes in respiration observed on stimulation are reflex effects.

2. The blood pressure is not governed by any special center in the thalamus; the changes observed are, as a rule, pressor effects.

3. Changes in pulse rate do not occur from moderate thalamic excitation.

4. There is no center controlling movements of the spleen; all changes are secondary to blood pressure changes.

5. The difference in respiratory effects noted on the stimulation of the thalamus and central grey are in all probability due to the stimulation of different paths going to the medullary centers.

6. The globus pallidus has an entirely different effect on the respiration from the thalamus and hence is connected with a different part of the medullary center.

7. The nucleus caudatus has no connection with the respiratory or circulatory mechanism.

8. The optic thalamus, nucleus caudatus, and nucleus lenticularis contain no centers which on direct stimulation produce changes in temperature.

In conclusion, I wish to express my thanks to Prof. C. G. L. Wolf for his valuable advice and helpful suggestions in the course of this investigation.

KEY TO FIGURES 1–6.

ST = make and break of current.
T = time marker.
C = Blood pressure.
R = Respiration.
S = Splenic curve.

Figures 8–11 are furnished to show the sections of brains that have been stimulated in the various lamellae.

KEY TO FIGURES 8–12.

ACQ = Anterior corpus quadrigeminum.
CG = Central grey.
F = Fillet.
FL = Fibers of Lancisi.
Fo = Fornix.
GH = Ganglion habenulae.
GP = Globus pallidus.
IC = Internal capsule.
My = Meynert's bundle.
NA = Nucleus anterior.
NC = Nucleus caudatus.
NL = Nucleus lateralis.
NLV = Nucleus lateralis ventralis.
NM = Nucleus medius.
NR = Nucleus reuniens.
PC = Posterior commissure.

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EXPLANATION OF PLATES.

Plate 38.

Fig. 1. Normal splenic curve. The lack of response upon stimulation of the fibers of Lancisi (G 12). The increase in respiratory change as the nucleus lateralis ventralis is approached (—B 12).

Fig. 2. Inspiratory inhibition upon globus pallidus stimulation. The circulation and spleen are unaffected.

PLATE 39.

FIG. 3. Normal splenic curve. Inert nucleus medius (C 10, O 10). Inspiratory inhibition from nucleus reuniens stimulation (—B 10). The same result is observed upon stimulation of the posterior commissure.

FIG. 4. Resemblance between hypothalamic stimulation (—B 8) and the globus pallidus (figure 2).

FIG. 5. Fornix (C 4), negative on stimulation. Fillet (O 4), great sensitiveness of this region; note the difference of the respiratory effect here and in figure 2.

PLATE 40.

FIG. 6. Great sensitiveness of the central grey (O 3, —A 3, —B 3). The first and third —A 3 show the result of subminimal current. Good normal spleen curve.

FIG. 7. Respiratory and circulatory changes from corpora quadrigemina, Türck's bundle, and the descending fifth root.

PLATES 41 TO 43.

FIGS. 8–12. Photographs showing the course of the needle track and the various anatomical points stimulated.

Experiment number.	Nucleus lateralis.			Nucleus lateralis ventralis.			Pulvinar (lateral).			Nucle
	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.
71...	Exp. inhib.	B. P. rose slightly	Nil	Exp. inhib.	B. P. rose slightly	Vol. increased slightly	.....	.....	.....	.....
73...	.....	.....	.....	.....	.....	.....	.....	.....	.....	Nil
75...	Exp. inhib.	B. P. rose slightly	Nil	.....	.....	.....	.....	.....	.....	Very slight slowing
77...	Exp. inhib.	B. P. rose slightly	Nil	.....	.....	.....	.....	.....	.....	Nil
79...	.....	.....	.....	Exp. inhib.	B. P. rose	Vol. increased slightly	.....	.....	.....	Nil
91...	Very slow	Nil	Nil	.....	.....	.....	.....	.....	.....	Nil
93...	Slower	Nil	Nil	.....	.....	.....	.....	.....	.....	.....
129...	.....	.....	.....	Slight slowing	Nil	Nil	Slightly slower	B. P. rose	Vol. increased	.....
131...	Rapid	B. P. rose	Nil	.....	.....	.....	.....	.....	.....	.....
133...	Slower	Nil	Nil	.....	.....	.....	.....	.....	.....	.....
135...	Slightly slower	Nil	Nil	.....	.....	.....	Exp. inhib.	B. P. rose slightly	Nil	.....
137...	Rapid	B. P. rose	Nil	.....	.....	.....	.....	.....	.....	.....
141...	Inspir. inhib.	Nil	Nil	.....	.....	.....	Nil	Nil	Nil	.....
143...	Slower	Nil	Nil	.....	.....	.....	.....	.....	.....	Nil
145...	Inspir. inhib.	B. P. fell	Nil	.....	.....	.....	.....	.....	.....	Nil
155...	Faster, shallow	Nil	Nil	.....	.....	.....	.....	.....	.....	.....
185...	Slight exp. inhib.	B. P. rose	Vol. decreased slightly	.....	.....	.....	.....	.....	.....	.....
187...	Slight exp. inhib.	B. P. rose	Vol. decreased	Slight inspir. inhib.	B. P. rose	Nil	.....	.....	.....	.....
189...	Slight exp. inhib.	B. P. fell	Nil	Exp. inhib.	B. P. rose	Vol. increased	Nil	Nil	Nil	Nil
191...	Exp. inhib.	B. P. rose	Vol. decreased	Inspir. inhib.	B. P. rose markedly	Nil	Nil	Nil	Nil	.....
193...	Exp. inhib.	Nil	Nil	Slight exp. inhib.	B. P. rose	Nil	Nil	Nil	Nil	Nil
195...	Exp. inhib.	Nil	Nil	Slight exp. inhib.	B. P. rose	Vol. increased	Nil	Nil	Nil	Nil
197...	Exp. inhib.	Nil	Nil	.....	.....	.....	Nil	Nil	Nil	Nil

Nucleus caudatus.			Nucleus lenticularis (globus pallidus).			Median fillet.			Fornix.			Nucleus medius			Pu
R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.
															Nil
Nil	Nil	Nil				Inspir. tetanus	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Exp. inhib.
Very slight slowing	Nil	Nil													
Nil	Nil	Nil							Nil	Nil	Nil				Exp. inhib.
Nil	Nil	Nil										Nil	Nil	Nil	Slightly more rapid
Nil	Nil	Nil													
			Inspir. inhib.	Nil	Nil										
												Nil	Nil	Nil	
			Inspir. inhib.	Nil	Nil										Exp. inhib.
			Inspir. inhib.	Nil	Nil										
															Slightly shallow
			Inspir. inhib.	Nil	Nil										
Nil	Nil	Nil													
Nil	Nil	Nil	Inspir. inhib.	Nil	Nil										
												Nil	Nil	Nil	
									Nil	Nil	Nil	Nil	Nil	Nil	
									Nil	Nil	Nil	Nil	Nil	Nil	Nil
Nil	Nil	Nil	Inspir. inhib.	B. P. rose markedly	Vol. increased	Inspir. tetanus	B. P. rose	Vol. increased							
			Inspir. inhib.	B P. rose. markedly	Vol. decreased	Inspir. inhib.	B. P. rose	Vol. decreased							
Nil	Nil	Nil	Inspir. inhib.	B. P. rose	Vol. decreased	Inspir. inhib.	B. P. rose	Nil	Nil	Nil	Nil				
Nil	Nil	Nil	Inspir. inhib.	B. P. rose	Vol. decreased	Inspir. tetanus	B. P. rose	Vol. increased	Nil	Nil	Nil				
Nil	Nil	Nil	Inspir. inhib.	B. P. rose	Vol. increased	Inspir. tetanus	B. P. rose	Nil	Slight slowing	B. P. rose	Nil				

R = respiration.
C = circulation.
S = spleen.

Inspir. inhib. = inspiratory inhibition.
Exp. inhib. = expiratory inhibition.
Vol. = volume.

E I.

inar (median).		Posterior corpus quadrigeminum.			Hypothalamus.			Türck's bundle.			Central grey	
C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.
Nil	Nil	.....	.....	.....	.....	.....	.....	Exp. inhib.	B. P. not recorded; violent movement	Vol. increased	.....	.....
Nil	Vol. increased slightly	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	Shallow and rapid	Nil	Nil	.....	.....	.....	.....	.....	.....	.....	.....
B. P. rose slightly	Vol. decreased slightly	Shallow and slightly slower	Nil	Nil	.....	.....	.....	.....	.....	.....	.....	.....
B. P. rose slightly	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	Slight exp. inhib.	B. P. rose	Nil	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	Slight exp. inhib.	Nil	Nil	.....	.....	.....	.....	.....
B. P. rose	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
B. P. rose	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	Exp. inhib.	B. P. rose slightly	Vol. increased	Exp. inhib.	B. P. rose	Vol. increased	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Rapid deep	B. P. rose
Nil	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	Rapid shallow tetanic	B. P. rose
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

B. P. = blood pressure { rose slightly = less than 5 mm. / rose = more than 5 and less than 15 mm. / rose markedly = more than 15 mm.

Türck's bundle.			Central grey.			Posterior commissure.			Ganglion habenulae and Meynert's bundle.			Center median and nucleus arcuatus		
R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	
Exp. inhib.	B. P. not recorded; violent movement	Vol. increased												
						Shallow and rapid	Nil	Nil						
												Very slow	B. P. rose	
Exp. inhib.	B. P. rose	Vol. increased												
			Rapid deep	B. P. rose	Vol. increased									
			Rapid shallow tetanic	B. P. rose	Vol. decreased				Exp. inhib.	B. P. rose	Vol. decreased			
						Exp. inhib.	B. P. rose	Nil						

s than 5 mm.
ore than 5 and less than 15 mm.
ore than 15 mm.

	Anterior corpus quadrigeminum.		
	R.	C.	S.
	Exp. inhib.	B. P. rose	Vol. decreased
	Exp. inhib.	B. P. rose	Nil

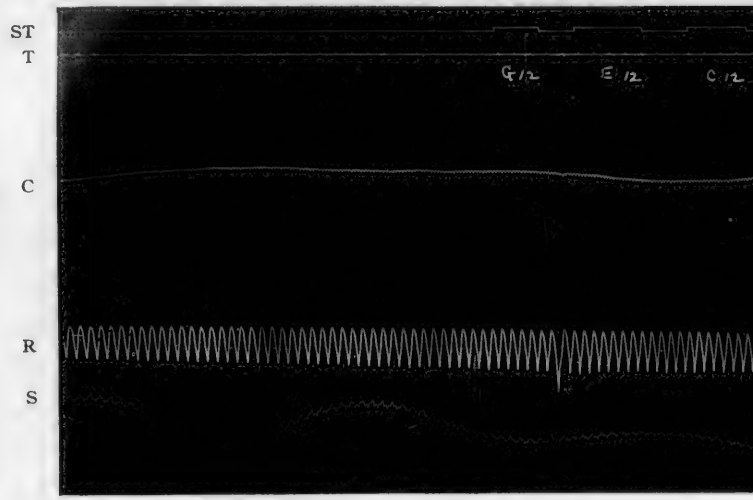
Experiment number.	Nucleus anterior.			Nucleus medius.			Nucleus reuniens.			Gangli
	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.
81...	Shallow	Nil	Nil	Nil	Nil	Nil	.....	.....	.....	.....
83...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
85...	Exp. inhib.	B. P. rose slightly	Vol. decreased	.....	.....	.....	.....	.....	.....	Exp inhi
87...	Exp. inhib.	B. P. rose slightly	Vol. decreased	Slightly slower	Nil	Nil	.....	.....	.....	.....
89...	Slow	B. P. rose slightly	Vol. decreased	.....	.....	.....	.....	.....	.....	Exp inhil
95...	Nil	B. P. rose markedly	Nil	.....	.....	.....	.....	.....	.....	Exp inhil
111...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
119...	.....	.....	.....	.....	.....	.....	.....	.....	.....	Rate increa
121...	Nil	B. P. rose	Nil	Nil	Nil	Nil	.....	.....	.....	.....
123...	.....	.....	.....	.....	.....	.....	.....	.....	.....	Exp inhil
139...	Slightly slower	B. P. rose markedly	Nil	.....	.....	.....	.....	.....	.....	.....
147...	Slightly shallower	B.P. rose markedly	Nil	.....	.....	.....	Inspir. inhib. tetanus	B. P. rose	Nil	.....
151...	.....	.....	.....	.....	.....	.....	Inspir. inhib. tetanus	B. P. rose	Nil	.....
153...	.....	.....	.....	.....	.....	.....	.....	.....	.....	Exp inhil
157...	Slightly slower	B. P. rose	Nil	.....	.....	.....	Inspir. tetanus	B. P. rose	Nil	.....
161...	.....	.....	.....	.....	.....	.....	Inspir. tetanus	B. P. rose	Nil	.....
163...	Inspir. inhib.	B. P. rose markedly	Vol. increased	.....	.....	.....	.....	.....	.....	Insp inhil
165...	Inspir. inhib.	B. P. rose markedly	Vol. increased	Nil	Nil	Nil	.....	.....	.....	Exp inhil
167...	Slight inspir. inhib.	B. P. rose	Nil	.....	.....	.....	Inspir. tetanus	B. P. rose	Nil	.....
169...	Inspir. inhib.	B. P. rose slightly	Nil	Nil	Nil	Nil	Inspir. tetanus	B. P. rose markedly	Vol. increased	.....
171...	Slight exp. inhib.	B. P. rose	Nil	Nil	Nil	Nil	Inspir. tetanus	B. P. rose markedly	Vol. increased	.....
173...	.....	.....	.....	.....	.....	.....	Inspir. tetanus	B. P. rose markedly	Vol. decreased	.....
175...	Inspir. inhib.	B. P. rose markedly	Vol. decreased	.....	.....	.....	.....	.....	.....	Exp inhi
177...	Nil	B. P. rose slightly	Vol. decreased	.....	.....	.....	.....	.....	.....	.....
179...	Nil	Nil	Nil	.....	.....	.....	Inspir. inhib.	B. P. rose markedly	Vol. decreased	.....
181...	.....	.....	.....	Nil	Nil	Nil	.....	.....	.....	Sligl inspi inhi
183...	.....	.....	.....	.....	.....	.....	Inspir. tetanus	B. P. rose	Nil	.....

n habenulae and Meynert's bundle.		Central grey.			Fibres of Lancisi.			Fornix.			Posterior corpus quad	
C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.
											Slight exp. inhib.	B. P. rose
		Slight inspir. tetanus	B. P. rose	Vol. decreased				Nil	Nil	Nil		
B. P. rose slightly	Nil							Nil	Nil	Nil		
					Nil	Nil	Nil					
B. P. rose slightly	Vol. decreased											
B. P. rose markedly	Vol. increased	Inspir. tetanus	B. P. rose	Nil				Nil	Nil	Nil		
		Inspir. tetanus	B. P. rose very slightly	Nil								
B. P. rose slightly	Nil	Inspir. tetanus	B. P. rose	Vol. increased markedly	Nil	Nil	Nil					
		Very rapid tetanus	B. P. rose	Nil	Nil	Nil	Nil					
B. P. rose slightly	Nil	Inspir. tetanus	B. P. rose	Vol. decreased								
		Inspir. tetanus	B. P. rose	Nil								
B. P. rose	Nil	Inspir. tetanus rapid, deep	B. P. rose	Vol. increased								
		Rapid shallow inspir. tetanus	B. P. rose	Vol. increased								
Nil	Nil	Inspir. tetanus	B. P. rose	Vol. increased	Nil	Nil	Nil	Nil	Nil	Nil		
B. P. rose	Vol. increased	Inspir. tetanus	B. P. rose	Vol. increased	Nil	Nil	Nil					
		Inspir. tetanus	B. P. rose	Vol. increased								
		Inspir. tetanus	B. P. rose	Vol. increased	Nil	Nil	Nil					
		Inspir. tetanus	B. P. rose slightly	Vol. increased								
		Inspir. tetanus rapid and below base line	B. P. rose	Vol. increased							Nil	Nil
B. P.rose	Vol. decreased	Inspir. tetanus rapid below base line	B. P. rose slightly	Vol. increased				Nil	Nil	Nil		
					Nil	Nil	Nil					
Nil	Nil	Inspir. tetanus below base line	B. P. rose	Nil	Nil	Nil	Nil					
		Inspir. tetanus shallow below base line	B. P. rose	Vol. decreased	Nil	Nil	Nil	Nil	Nil	Nil		

TABLE II.

nix.		Posterior corpus quadrigeminum.			Anterior corpus quadrigeminum.			Posterior commissure.			Nucleus caudatus.			Posterior lo bun	
.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C	S.	R.	C
...	.....	Slight exp. inhib.	B. P. rose	Vol. decreased	.....	.....	.....	Exp. inhib.	B. P. rose	Vol. decreased	Nil	Nil	Nil	.....	...
il	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Exp. inhib.	Ni
il	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	Exp. inhib.	Nil	Nil	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
il	Nil	.....	.....	.....	Slightly more rapid	B. P. rose	Vol. increased	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	Nil	Nil	Nil	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Exp. inhib.	B. ros
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	Shallow and rapid	B. P. rose	Vol. increased	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	Shallow then rapid and deep	B. P. rose	Nil	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	Exp. inhib.	B. P. rose	Nil	.....	.....	.....	.....	...
il	Nil	.....	.....	.....	.....	.....	.....	Exp. inhib.	B. P. rose	Vol. decreased	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	Exp. inhib.	B. P. rose markedly	Vol. increased	.....	.....	.....	.....	...
...	.....	.....	.....	.....	Shallow then rapid	B. P. rose	Vol. decreased	Exp. inhib.	B. P. rose slightly	Nil	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	Nil	Nil	Nil	.....	.....	.....	Exp. inhib.	B. P. rose	Vol. decreased	.....	.....	.....	.....	...
il	Nil	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...
il	Nil	.....	.....	.....	Shallow, rapid	B. P. rose	Vol. decreased	Exp. inhib.	B. P. rose	Vol. decreased	.....	.....	.....	.....	...

gitudinal e.		Descending 5th root.			Pulvinar (median).			Globus pallidus.			Nucleus lateralis ventralis.			Hypothalamus.		
	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.	R.	C.	S.
	Nil															
		Exp. inhib.	Nil	Nil												
	Nil										Exp. inhib.	B. P. rose markedly	Nil			
					Exp. inhib.	B. P. rose	Nil				Shallow	B. P. rose	Nil			
								Inspir. inhib.	B. P. rose	Nil						
											Slight exp. inhib.	Nil	Nil			
														Exp. inhib.	B. P. rose	Nil
								Inspir. inhib.	B. P. rose slightly	Nil						
											Slightly shallow	B. P. rose	Nil			
											Exp. inhib.	Nil	Nil			
											Exp. inhib.	Nil	Nil			
					Exp. inhib.	B. P. rose	Nil				Exp. inhib.	B. P. rose	Nil			


ST
T
C
R
S

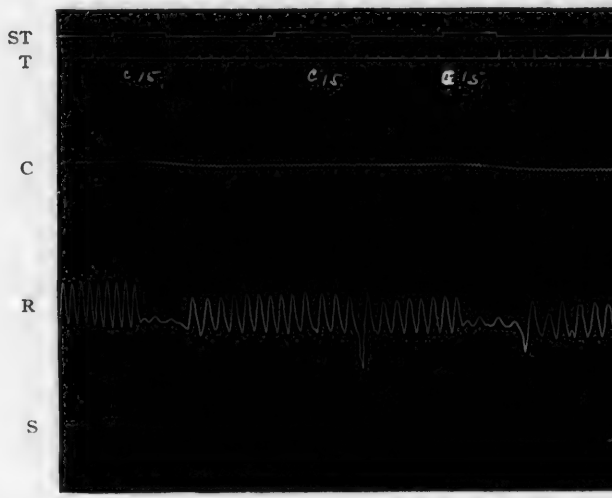

ST
T
C
R
S

PLATE 38.

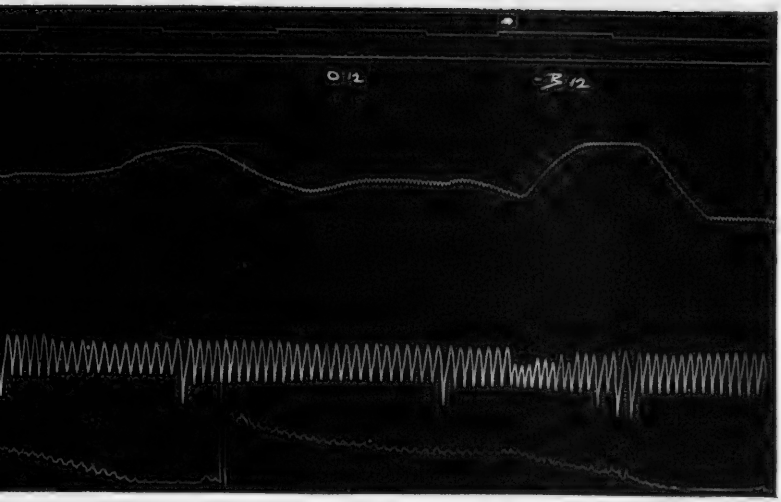


FIG. 1.

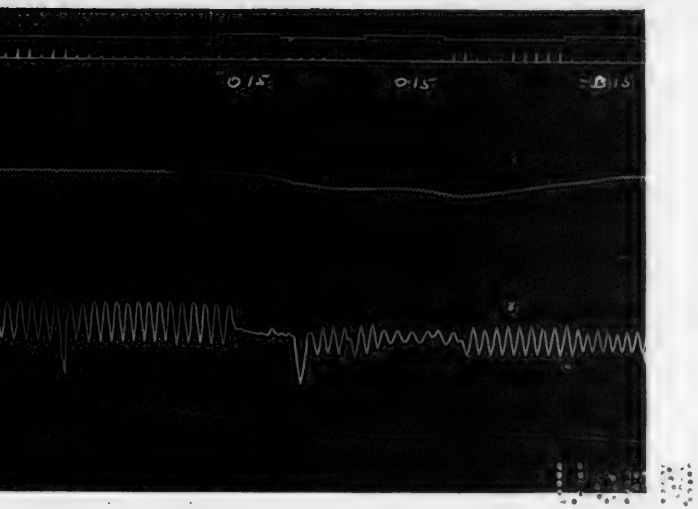
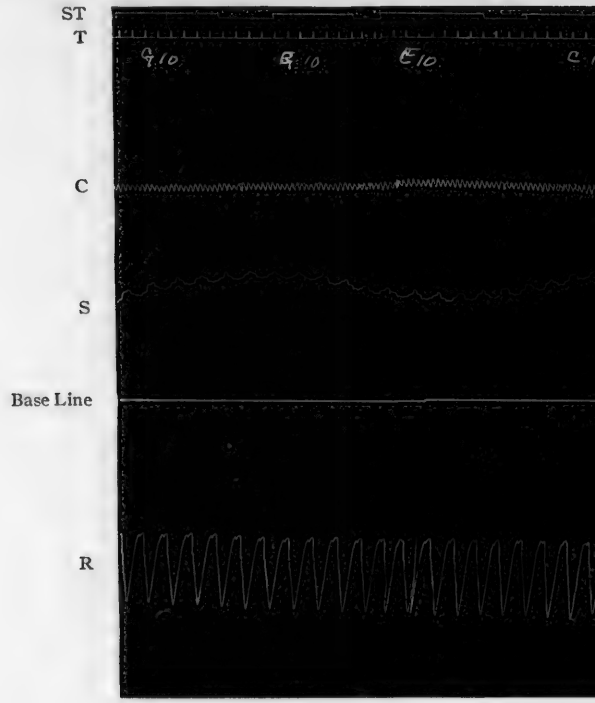


FIG. 2.



Fi

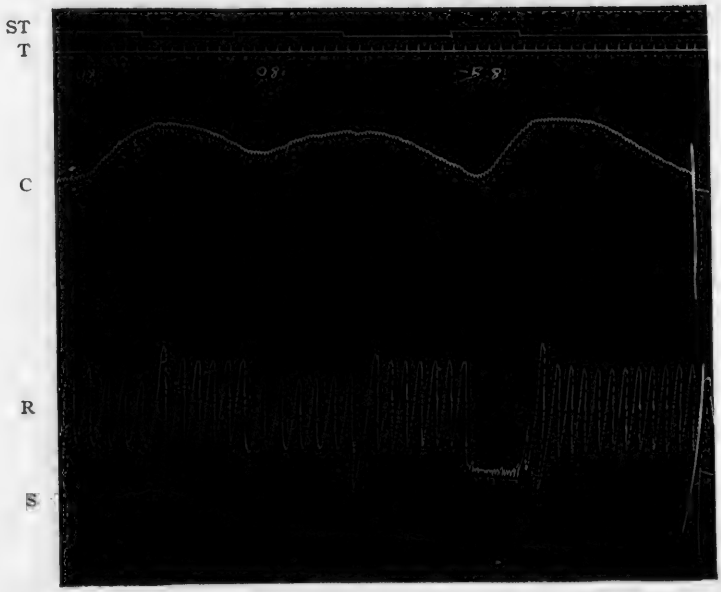


FIG. 4.

3.

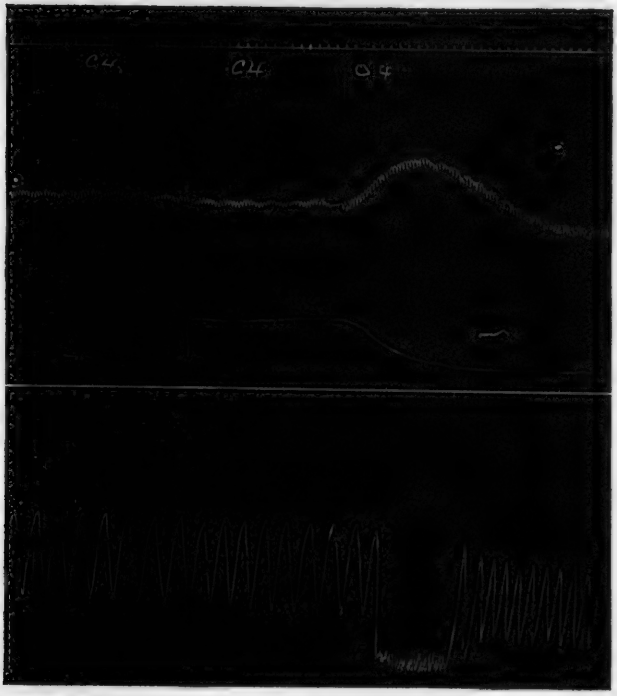


FIG. 5.

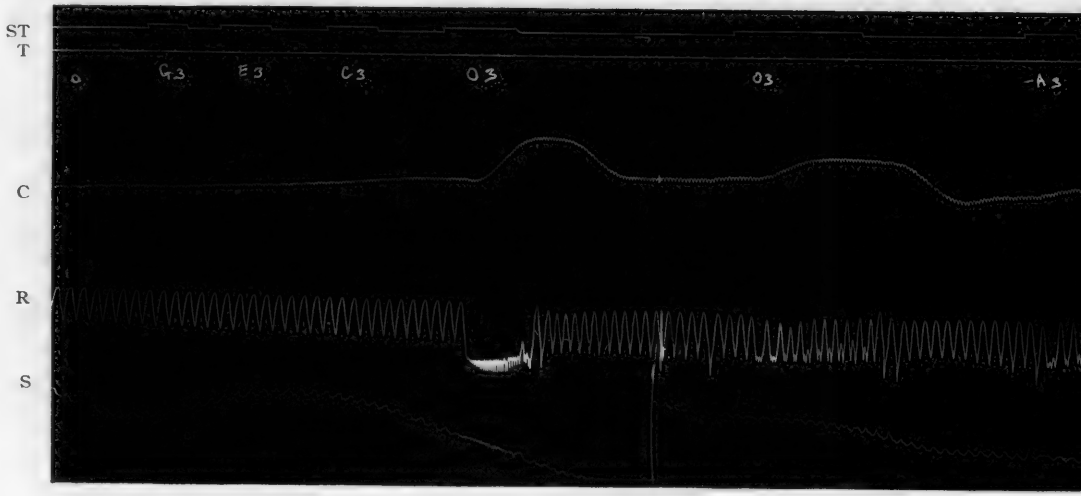


FIG. 6

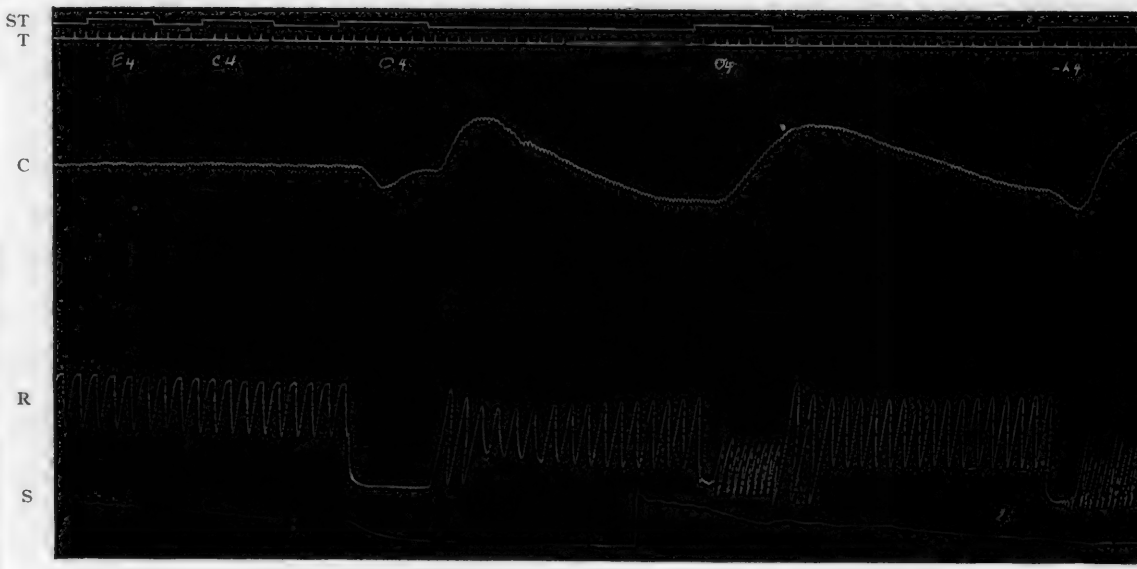
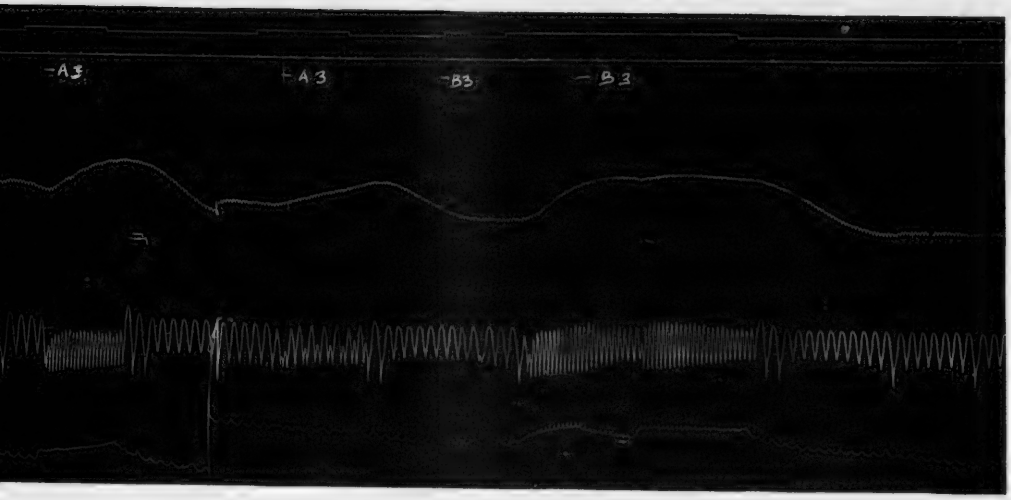
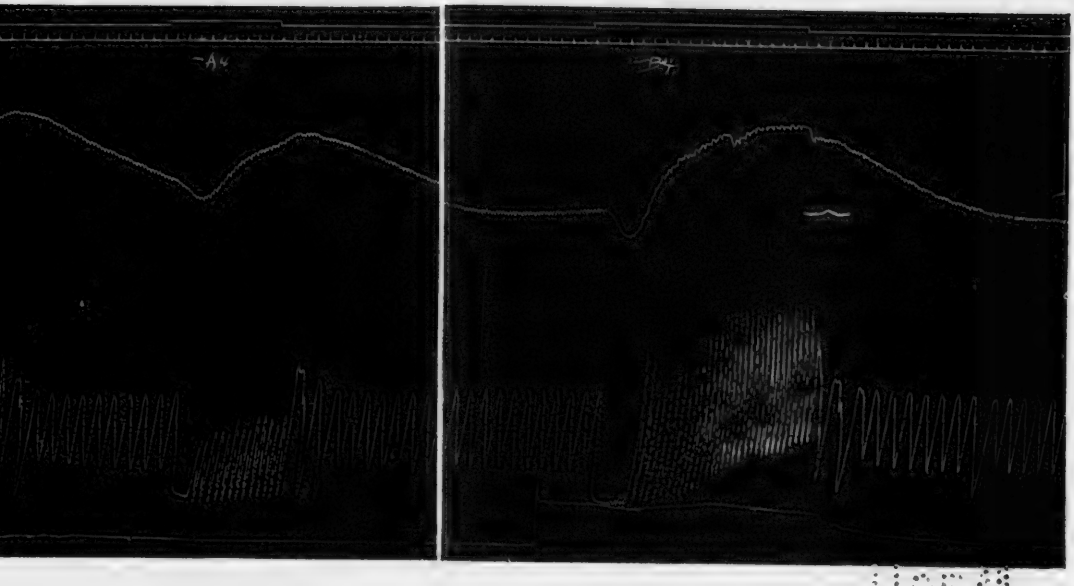


FIG. 7

PLATE 40.





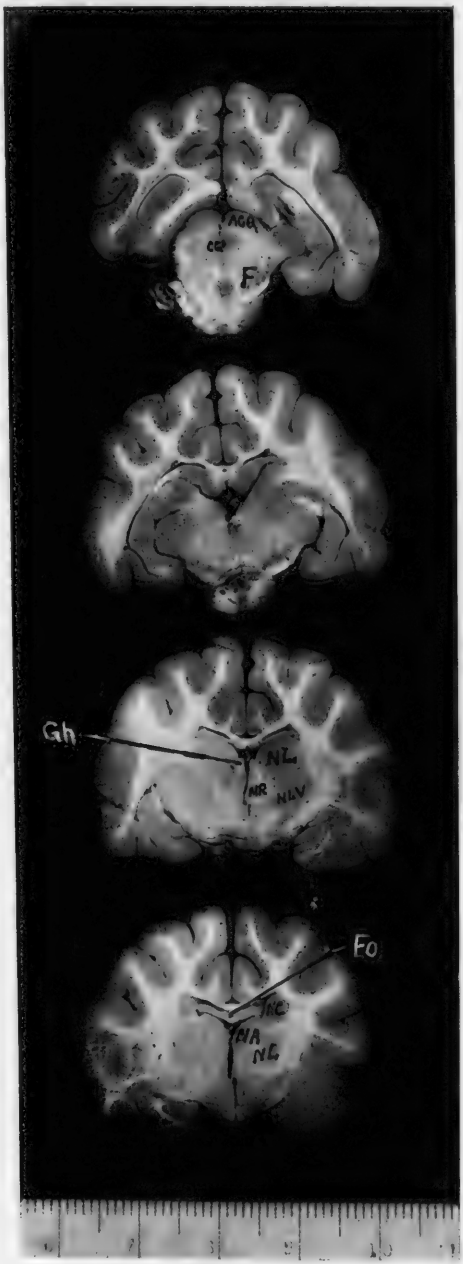


FIG. 8.

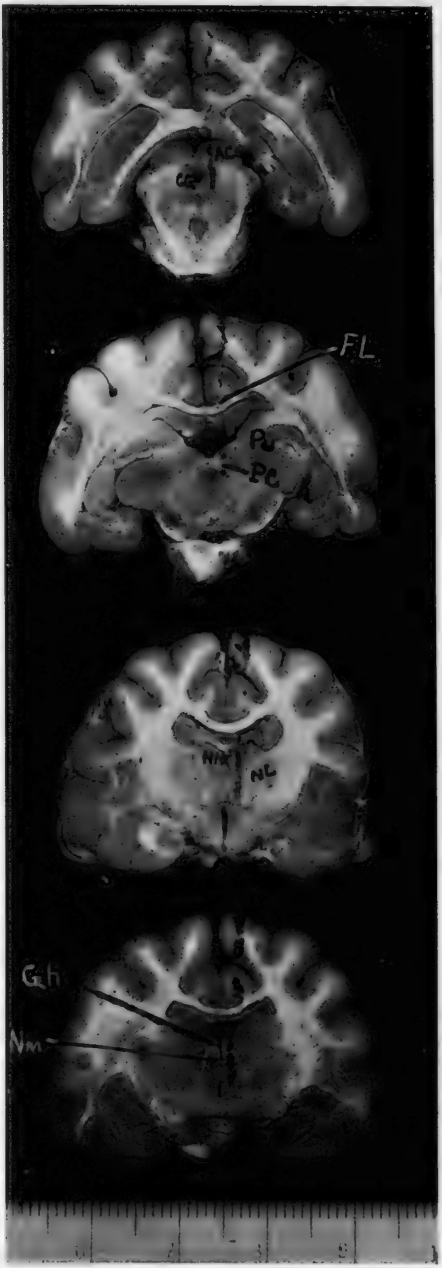


FIG. 9.

FIG. 10.

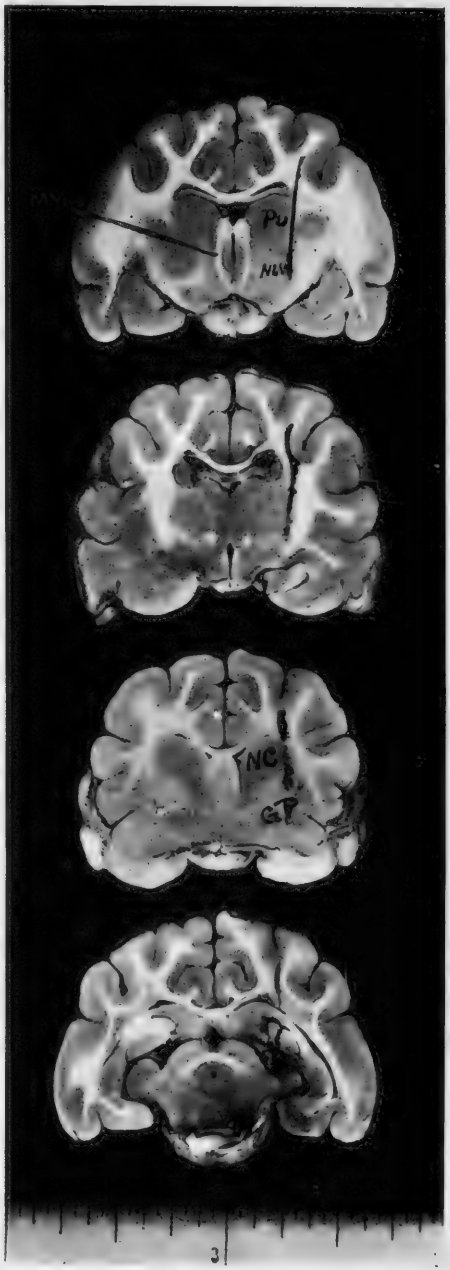


FIG. 11.

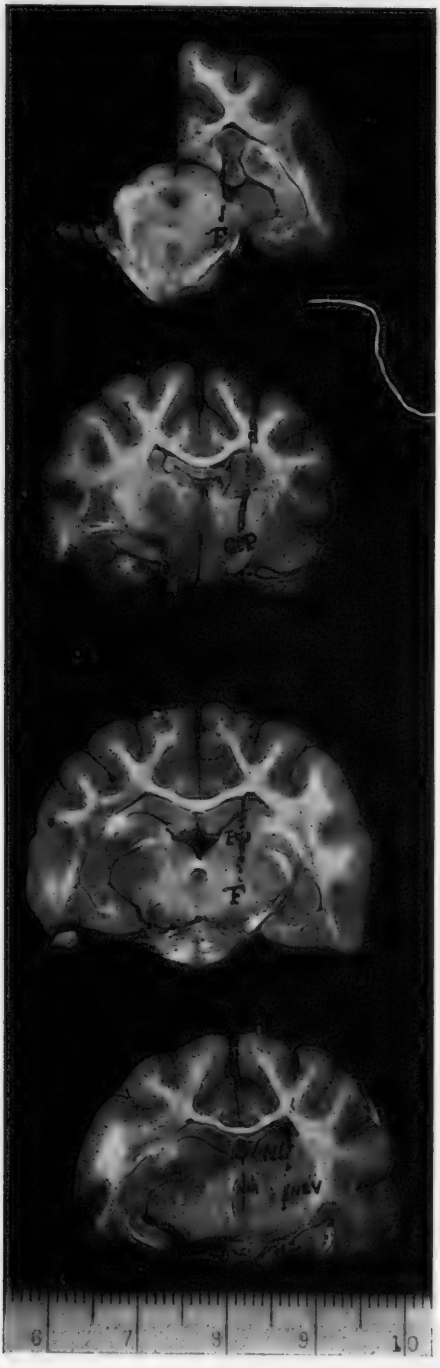


FIG. 12.

A METHOD FOR THE QUANTITATIVE DETERMINATION OF FECAL BACTERIA.*

By H. A. MATTILL and P. B. HAWK.

(*From the Laboratory of Physiological Chemistry of the University of Illinois, Urbana.*)

The composition of feces, both of animals and of man, during fasting and on many different diets, has been determined frequently, but despite the many investigations there are widely different statements as to the nature and probable source of the substances found. The apparent uniformity in kind and quantity of fecal material resulting from different diets is a striking fact and has been explained in several different ways: that the feces are chiefly the unabsorbed residues of intestinal excretions (1) which might naturally vary but little, or that the feces consist chiefly of the bodies of dead and living bacteria (2), whose amount might also be fairly constant. Probably neither of these views is correct. It was shown by Strasburger (3) that ordinarily about one third of the dry substance of the feces consists of microörganisms, and that from one fourth to one half of the fecal nitrogen is bacterial in its origin.

The importance of the bacterial flora in the intestinal tract has been emphasized in a recent investigation by MacNeal, Latzer, and Kerr (4), and in investigations, reported almost simultaneously with this, by Sato (5) and by Berger and Tsuchiya (6). The first mentioned investigators examined 266 stools of twelve men, on a normal mixed diet, during a prolonged metabolism experiment. The results include microscopic differential counts by several methods, gravimetric determinations of bacterial substance and determinations of the ratio of bacterial nitrogen to total fecal nitrogen. By MacNeal's modification of Strasburger's method of fractional sedimentation, these investigators found the average daily output of dry bacteria to be 5.34 gm., the average daily bacterial dry substance to be 26.9 per cent. of the fecal dry substance, and the average daily bacterial nitrogen 46.3 per cent. of the total fecal nitrogen. They

* Presented in abstract at the New Haven meeting of the American Society of Biological Chemists, December, 1910. Received for publication, June 29, 1911.

conclude that direct quantitative determinations of fecal bacteria furnish evidence of the extent and nature of the bacterial growth in the intestine, and that this seems to be a delicate index of intestinal conditions.

In the opinion of Sato (5), an examination of the feces is as important as that of the stomach contents, perhaps even more so. He examined thirty consecutive stools (mixed diet) by the method of Strasburger and found an average daily excretion of dry bacteria equal to 8.54 gm. and an average of 24.39 per cent. of dry bacteria in dry feces.

By the same method, the results of Berger and Tsuchiya (6) show very much smaller values for these quantities; an average daily excretion of 3.023 gm. dry bacteria, which form 12.6 per cent. of the dry feces. Tobaya (7) found a value of 11.22 per cent. for this ratio. The last mentioned workers consider the lower results to be more accurate than those of Sato, Strasburger, and others, because these investigators employed hand centrifuges, which are less rapid in their action and less easily maintained under uniform control. None of these last mentioned investigations include data on the nitrogen content of bacteria.

There are several methods for the quantitative determination of bacterial substance gravimetrically, or rather several modifications of Strasburger's method of fractional sedimentation (2). According to the technique originally used by this investigator, 2 c.c. of a fecal suspension are subjected to two serial centrifugalizations to remove the non-bacterial solid material. The method of Steele (8) involves a single centrifugalization of 5 c.c. of a fecal suspension, followed by filtration through muslin to remove the remaining non-bacterial solid matter. The method of MacNeal (4) begins with a 2 to 3 gm. sample of fresh feces, which is brought into suspension in 0.2 per cent. hydrochloric acid in a centrifuge tube and subjected to three serial centrifugalizations, or four if necessary.

PURPOSE AND CONDITIONS OF THE EXPERIMENTS.

In the belief that the quantity of intestinal bacteria would furnish evidence as to the comparative utilization, particularly of the protein material of the food, under different conditions, it was deemed advisable to determine the nitrogen content of the bacterial portion of each individual stool obtained throughout several metabolism experiments. The object of these experiments was to discover the influence of varying amounts of water taken with the meals upon the digestion and absorption of food. Since the diet of the subjects of these experiments was absolutely uniform from meal to meal, any variation in the bacterial content of the feces could not be attributed to the effect of different diets.

The subjects of the experiments and their diet and daily routine have been more fully described elsewhere.[1] The daily periods began and ended at 7 A. M. after urinating and defecating. The three

[1] Mattill and Hawk, *Jour. Am. Chem. Soc.*, 1911, xxxiii.

meals were taken as regularly as possible each day at 7:30, 12:15, and 5:30; the meals were identical and consisted of graham crackers, butter, peanut butter, milk, and water, a diet containing but small amounts of cellulose.

Each stool was passed into a weighed porcelain dish which was then weighed again. The consistency of the stool determined the ease with which a uniform mixture could be obtained. Bone spatulas proved to be very satisfactory for breaking up and crushing the larger masses. This was done as quickly as possible to prevent loss by evaporation, which is very rapid. In obtaining the samples for analysis, the use of glass-stoppered weighing bottles and small porcelain spatulas was found most satisfactory. Weighing by difference was more accurate as well as more rapid than by the direct method.

BACTERIAL NITROGEN. THE SEPARATION OF BACTERIA FROM FECES.

The method which was adopted for this procedure and determination is a simplification of that of MacNeal. About two grams of feces are accurately weighed and placed in a fifty cubic centimeter centrifuge tube. To the feces in the tube a few drops of 0.2 per cent. hydrochloric acid are added, and the material is mixed to a smooth paste by means of a glass rod. Further amounts of the acid are added with continued crushing and stirring until the material is thoroughly suspended. The tube is then whirled in the centrifuge at high speed for one half to one minute. The suspension is found sedimented into more or less definite layers, the uppermost of which is fairly free from the larger particles. The upper and more liquid portion of the suspension is now drawn off by means of a pipette and transferred to a beaker.[2] The sediment remaining in the tube is again rubbed up with the glass rod with the addition of further amounts of dilute acid, and again centrifugalized for one half to one minute. The supernatant liquid is pipetted off and added to the first, the same pipette being used for

[2] A 25 cubic centimeter pipette is the most satisfactory size; to facilitate observation, the delivery tube is bent near the bulb to an angle of about 120 degrees.

the one determination throughout.[3] A third portion of the dilute acid is then added to the sediment, which is again mixed by stirring and again centrifugalized. All the washings are added to the first one, and during the process care is taken to wash the material from the walls and mouth of the centrifuge tube down into it. Finally, when the sediment is sufficiently free from bacteria, the various remaining particles are visibly clean, and the supernatant liquid after centrifugalization remains almost clear. This is removed to the beaker in which are now practically all the bacteria present in the original portion of feces, together with some solid matter not yet separated. In the centrifuge tubes there is a considerable amount of bacteria-free solid matter. This sediment and the others following are all combined in a Kjeldahl flask and their nitrogen content is determined. It has been called the residue nitrogen, and it represents undigested and insoluble nitrogen.

The suspension is now transferred to the same centrifuge tube, centrifugalized for a minute, and the supernatant liquid transferred to a clean beaker by means of the same pipette. The tube is then refilled from the first beaker and thus all the suspension centrifugalized a second time. The beaker is finally carefully washed with the aid of a rubber-tipped glass rod, the second sediment in the centrifuge tube is washed free of bacteria by means of this wash water and by successive portions of the dilute acid, and the supernatant liquid after centrifugalization is added to the contents of the second beaker. The second clean sediment is added to the first. The bacterial suspension now in the second beaker is again centrifugalized in the same way and a third portion of bacteria-free sediment is separated. Frequently a fourth serial centrifugalization is performed,—always if the third sediment is of appreciable quantity. At all stages of the separation, small portions of the dilute hydrochloric acid are used, so that the final suspension shall not be too voluminous. Ordinarily it amounts to 125 to 200 cubic centimeters. At the same time, the final amount of fluid should not be too small, as shown by Ehrenpfordt (9), because the vis-

[3] A convenient support for the pipettes is a wire spring on a glass base, such as is used on a desk for pen-holders. The delivery tube, just where it is bent, is inserted between the wires, and any liquid not delivered collects in the bend of the tube.

cosity accompanying increased concentration prevents proper and complete sedimentation.

To the final bacterial suspension an equal volume of alcohol is added and the beaker set aside to concentrate. A water bath at 50° to 60° C. is very satisfactory. After two or three days, when the liquid is concentrated to about fifty cubic centimeters, the beaker is removed and about 200 cubic centimeters of alcohol are added. The beaker is covered and allowed to stand at room temperature for twenty-four hours. At the end of this time the bacterial substance is generally settled, so that most of the clear supernatant liquid, of dark brown color, can be directly siphoned off without loss of solid matter. The remainder is then transferred to centrifuge tubes, centrifugalized, and the remaining clear liquid pipetted off. The sediment consists of the bodies of the bacteria, and is transferred to a Kjeldahl flask for nitrogen determination. This is the bacterial nitrogen. Where a determination of bacterial dry substance is desired, the sediment of bacteria is extracted by absolute alcohol and ether in succession, transferred to a weighed porcelain crucible, and dried at 102° C. to constant weight. This dried sample is then used in the nitrogen determination. Our procedure differs from that of MacNeal in that the bacterial dry matter is not determined. A saving of about seven days' time and of considerable labor is accomplished by this omission.

The alcohol which is siphoned off, the last portions of which are separated from the bacteria by centrifugalization, contains such nitrogen as has been extracted from the bacteria and other fecal material, and, in addition, all fecal nitrogen which is soluble in 0.2 per cent. hydrochloric acid or which has become so during the time of manipulation. This comprises all unabsorbed soluble digestion products, the soluble nitrogen of the digestive juices and bile pigments, and the nitrogenous extractives of the bacteria. The alcohol precipitate may contain, in addition to bacterial substance, protein material which is not bacterial in its origin, such as mucin, and the application of this method to pathological stools of such a nature that the amount of this substance is increased, would involve a correction on this account. This is true for all the methods in which a precipitation by alcohol is employed. In his

investigation upon the accuracy of Strasburger's method, Ehrenpfordt (9) attempted, by various means, to eliminate the soluble protein precipitable by alcohol, but he was not successful. He suggests that the final large dilution of an originally small sample would practically eliminate this source of error.

When the extraction by alcohol was not performed and nitrogen was determined directly on the supernatant fluid after the last sedimentation, the amount of nitrogen was considerably higher. It was, in fact, but little below the total fecal nitrogen and bore a constant ratio to it. This shows that the alcohol extraction does remove nitrogenous substances,—an action which MacNeal, Latzer, and Kerr (10) have thought to be not impossible, although they say the amount of nitrogen in dry bacterial substance is ten to fifteen per cent. greater when calculated upon extracted residue. Since the method herein described does not include a determination of dry bacterial substance, it is not possible to show from the data whether this is true here. Most frequently, in investigations upon bacterial proteins, the substance employed is the bacterial residue after extraction with alcohol and ether.

The same investigators (10) found the nitrogen content of the dry bacterial residues to vary from ten to twelve per cent. with an average of 10.96 per cent., a value which they verified on pure cultures, and which agrees with the values found by other investigators, among them Cramer (11) and Leach (12). In an examination of pure cultures prepared on a large scale, Nicolle and Alilaire (13) found the nitrogen content of *Bacillus coli* to be 10.32 per cent. In view of the susceptibility of microörganisms and their prompt adaptation to their surroundings, results obtained under other than natural conditions have little value in this connection. A value of 10.97 per cent. for the avérage nitrogen content of dried extracted fecal bacteria was obtained by Fowler and Hawk (14), who used the method of MacNeal for the determination. Inasmuch as it has been shown by various investigators that such bacteria as are present in the feces contain on the average about 11 per cent. of nitrogen, the values for bacterial nitrogen as determined by our method may conveniently serve as a basis for the calculation of the actual output of bacterial substance.

For the following tables, which include data on total fecal nitrogen and on bacterial nitrogen by our method, we have calculated the bacterial dry matter of each stool on the basis of a nitrogen content of 10.96 per cent. Also for purposes of comparison, the amount of dry bacterial substance in fecal dry substance has been calculated.

DISCUSSION OF RESULTS.

Of the sixty-six stools completely examined in this way, the data on twenty-three are given as representative. The averages are on the basis of the fecal excretion per day throughout the entire period.

TABLE I.

Bacterial Nitrogen. Subject W.

Number of stool.	Weight of stool in gm.	Per cent. dry matter.	Amount dry matter in gm.	Fecal nitrogen in gm.	Bacterial nitrogen in gm.	Bacterial dry substance (calculated) in gm.	Per cent. dry bacteria in dry feces (calculated).	Per cent. bacterial nitrogen in fecal nitrogen.
7	62.8	25.61	16.1	0.845	0.483	4.404	27.49	57.2
8	98.8	26.72	26.4	1.265	0.693	6.319	23.93	54.8
11	41.8	31.23	13.1	0.673	0.287	2.616	20.05	42.6
12	104.9	24.28	25.5	1.254	0.590	5.378	21.11	47.0
15	147.5	27.52	40.6	2.044	0.984	8.977	22.12	48.1
16	75.4	26.20	19.8	1.029	0.563	5.131	25.97	54.7
17	144.8	27.45	39.8	2.044	1.087	9.923	24.96	53.2
21	169.0	26.52	44.8	2.355	1.298	11.84	26.43	55.1
27	142.7	25.05	35.8	1.845	1.160	10.58	29.57	62.9
28	81.4	27.96	22.8	1.238	0.758	6.914	30.33	61.2
31	136.0	25.5	34.6	1.895	1.072	9.775	28.24	56.6
Average daily excretion of dry bacteria						6.97	26.0	54.1

In the data of table I, subject W, the calculated percentage of dry bacteria in dry feces varies from 20.05 per cent. to 30.33 per cent., with an average value of 26.0 per cent. The percentage of fecal nitrogen found existing as bacterial nitrogen varies from 42.6 per cent. to 62.9 per cent., with an average of 54.1 per cent.

The data of table II, subject E, show the calculated percentages of dry bacteria in dry feces to vary from 24.45 per cent. to 35.29 per cent., with an average of 29.9 per cent. The percentage of fecal nitrogen existing as bacterial nitrogen varies from 45.4 per cent. to 61.5 per cent., the average being 53.7 per cent.

A closer examination of the tables reveals no relation between the percentage of dry matter and the percentage of dry bacteria in dry

TABLE II.

Bacterial Nitrogen. Subject E.

Number of stool.	Weight of stool in gm.	Per cent. dry matter.	Amount dry matter in gm.	Fecal nitrogen in gm.	Bacterial nitrogen in gm.	Bacterial dry substance (calculated) in gm.	Per cent. dry bacteria in dry feces (calculated).	Per cent. bacterial nitrogen in fecal nitrogen.
7	124.6	22.30	27.8	1.744	0.987	9.005	32.36	56.6
11	63.8	29.16	18.6	1.158	0.685	6.250	33.61	59.2
12	169.0	24.30	41.1	2.577	1.372	12.52	30.45	53.3
14	135.3	22.96	31.1	1.896	1.019	9.290	29.87	53.8
15	192.4	19.58	37.7	2.389	1.083	9.880	26.21	45.4
16	79.2	24.58	19.5	1.144	0.523	4.769	24.45	45.7
22	147.7	23.19	34.3	2.045	1.068	9.742	28.40	52.2
b 1	35.2	28.88	10.2	0.572	0.307	2.799	27.45	53.7
b 3	202.2	24.65	49.8	3.036	1.715	15.64	31.41	56.5
b 7	34.6	27.24	9.4	0.591	0.364	3.317	35.29	61.5
b12	258.0	14.81	38.2	2.250	1.081	9.860	25.81	48.1
b17	50.6	11.06	5.6	0.340	0.159	1.454	25.96	47.0
Average daily excretion of dry bacteria						9.57	29.9	53.7
Average daily excretion of dry bacteria, subject W (table I)						6.97	26.0	54.1
Average daily excretion of dry bacteria, subjects W and E						8.27	27.95	53.9

feces, but such a relation is highly improbable, since it has been shown that bacteria do not possess a typical water content (15), (16), (17). With but a few exceptions, however, the percentage of dry bacteria in dry feces varies in the same way as the percentage of bacterial nitrogen in fecal nitrogen. This means that on a uniform diet the bacterial nitrogen of the feces varies, in general, in the same way as the amount of dry matter in the feces. The same statement can also be made regarding the fecal nitrogen in its relation to total dry matter. It is thus shown to be unnecessary to obtain the dry bacteria as a preliminary step in the determination of their nitrogen content.

Combining the data from both tables, the bacterial dry substance constitutes 20.05 to 35.29 per cent. of the fecal dry substance, with an average of 27.95 per cent. MacNeal, Latzer, and Kerr (18) found the variation to be from 14.03 to 42.53 per cent., with an average value of 26.9 per cent. dry bacterial substance in dry feces, a value about 1 per cent. lower than our own. The combined data show a variation of 42.6 to 62.9 per cent. for the portion of bacterial fecal nitrogen, the average value being 53.9 per cent. The investigators just mentioned (19) found the variations in this quan-

tity to be from 23.3 to 66.8 per cent., with an average of 46.3 per cent. While our values show smaller variations, they are uniformly higher, the average being 7.6 per cent. more than in the investigations of MacNeal, Latzer, and Kerr.

Since in the determination of dry bacterial substance, a factor obtained by other investigators was used, our results upon the daily output of dry bacterial substance have not the value of original determinations, although from the uniform results of several investigators upon the nitrogen content of dry bacteria there seems to be a reasonable basis for calculation. Among the determinations of the daily excretion of dry bacteria are the following (previously mentioned):

	Grams.
Strasburger	8.0
Sato	8.54
Berger and Tsuchiya	3.023
MacNeal, Latzer, and Kerr	5.34
Mattill and Hawk	8.27

The data on the percentage of dry bacteria in dry feces include the following:

	Per cent.
Strasburger	24.3
Schittenhelm and Tollens[4]	42.0
Lissauer[4]	8.67
Tobaya	11.22
Sato	24.39
Berger and Tsuchiya	12.6
MacNeal, Latzer, and Kerr	26.9
Mattill and Hawk	27.95

These widely different values have all been obtained by Strasburger's method, or by modifications thereof. It is claimed by Sato (5), as well as by Ehrenpfordt (9), that in normal individuals passing normal stools, diet has no appreciable effect on the quantity of bacteria excreted. All the variations shown above are caused, according to Ehrenpfordt (9), by differences in the centrifugalization procedure. A two minute centrifugalization causes non-bacterial matter to be left in suspension, and gives high results

[4] Quoted by Ehrenpfordt, *loc. cit.*

(Strasburger, Sato), while a ten minute centrifugalization by a precipitation of considerable bacteria along with the non-bacterial matter causes low results (Tobaya, and Berger and Tsuchiya). Ehrenpfordt claims that an absolute separation by centrifugalization is not possible, but that the more accurate values probably lie between those given and are more nearly obtained by an intermediate centrifugalization time, *i. e.*, about five minutes.

It should be emphasized in this connection that the technique of serial centrifugalization, in which a two minute period is used, will, if properly conducted, obviate to a large degree the difficulty mentioned by Ehrenpfordt. Furthermore, the fact that different observers do not agree does not invalidate the gravimetric method as compared with the counting methods, and a given procedure in the hands of one observer yields entirely comparable results.

Notwithstanding the negative conclusions regarding the effect of diet, a possible reason for our uniformly higher results in the percentage of bacterial nitrogen in fecal nitrogen is suggested by the findings of Herter and Kendall (20). These investigators found in experiments on kittens and monkeys that frequent alternations in the chemical nature of the diet were beneficial, since they interfered with the establishment of any one type of bacteria in the intestine and thereby diminished intestinal putrefaction. The subjects of our experiments were on an absolutely uniform diet both as to kind and quantity of food.

In addition to a possible uniform difference in the procedure of sedimentation, it is to be recalled that Strasburger's original method, as well as most of its modifications, includes alcohol and ether extraction, while our method involves only an alcohol extraction. Any ether-soluble nitrogenous substances, such as lecithin and other lipoids, would not be extracted as completely by our method as by the other methods, and without any values as to the weight of our dried bacteria it can not be stated whether the nitrogen values on the basis of dry bacteria are greater or less when these are extracted merely with alcohol, than when both alcohol and ether are used.

Since bacterial nitrogen is desired, it seems more reasonable to avoid as much as possible the removal by solvents of such nitrog-

enous substances as are normal constituents of bacteria. On this basis the higher values obtained by our method are probably nearer the true value for "bacterial" nitrogen than where the organisms are subjected to ether extraction.

This method will also be applied to the determination of bacteria in the feces of herbivora, where the diet, although not so completely available contains a large amount of cellulose.

SUMMARY.

From results of which the foregoing data form a part, it appears that the amount of bacterial nitrogen in the feces is a valuable index to intestinal conditions, and the method herein described is a simple and satisfactory one for making this determination. It involves three serial centrifugalizations of a two gram sample of the fresh feces brought into suspension in 0.2 per cent. hydrochloric acid. The bacterial suspension finally obtained is concentrated and extracted by alcohol, and nitrogen is determined in the precipitated material. The complete data on a given stool can be obtained in about five days, and one operator can take care of three or four stools in duplicate in one day.

On an absolutely uniform diet of simple and easily digested food during a period of three to four weeks, the average amount of bacterial nitrogen in two subjects was found to be 53.9 per cent. of the total fecal nitrogen, and this percentage, though higher than that obtained by workers heretofore, is probably more nearly a true value for bacterial nitrogen, because no ether extraction was employed.

The average daily amount of dry bacteria, calculated on the basis of the nitrogen values, is 8.27 grams.

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